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PHYTOPATHOLOGY

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VARIABILITY IN THE FIRE-BLIGHT ORGANISM, ERWINIA AMYLOVORA[1]

P. A. Ark

(Accepted for publication December 9, 1935)

Following the discovery of *Erwinia amylovora* (Burrill) Comm. S.A.B., the cause of fire blight, the pathogen was studied culturally by a number of investigators. Among these there appears to be a disagreement as to certain major and minor characteristics of the organism, although methods and media used were apparently identical in many instances. Variation among isolates of the organism has been touched upon by various workers (29, 45, 46) and studied more specifically by a few (27, 41) without, however, their having reached a final conclusion as to the degree or cause of variation.

In an attempt to find a reasonable explanation for differences among cultures reported by others or observed by the writer, 10 different isolates of *Erwinia amylovora* were selected representing 6 different localities and 8 different hosts, and these, after being single-celled by the method of Avery and Leland (5), were studied under different standard and special laboratory conditions. The source of the isolates is shown in table 1.

MORPHOLOGY

Size of Cells. The isolates showed considerable variation in behavior on various laboratory media. Bacteria were found to vary in size regardless of whether taken from the infected parts direct or from cultures. To study variation in size the organisms were passed by inoculation through a pear shoot 3 times, reisolated, and then grown for 24 hours at 28° C. on nutrient meat-extract agar adjusted to pH 6.9. Smears were made from the water of condensation in an agar tube and stained by Gram's method. Five

[1] This is abridged from a thesis presented to the Graduate Division of the University of California in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

The writer wishes to express his appreciation to Dr. H. Earl Thomas and Prof. Ralph E. Smith for their advice during the investigation and for aid in preparation of the manuscript.

Contribution from the Division of Plant Pathology, University of California, Berkeley, California.

TABLE 1.—*Source of isolates*[a] *of Erwinia amylovora*

Laboratory number	Isolated from	Part of the plant	Date of isolation	Geographical locality
50	Pyracantha angustifolia	Stem (canker)	Aug. 28, 1929	Berkeley, California
55	P. angustifolia	Berry	Jan. 4, 1932	Berkeley, California
57	P. coccinea lalandi	Blossoms	May 25, 1930	San Jose, California
64	Photinia arbutifolia	Blossoms	Sept. 10, 1931	Berkeley, California
77S[b]	Pyrus malus		Sept., 1915	Unknown
500–B	Crataegus monogyna	Twig	July, 1931	Ithaca, New York
501	Crataegus oxyacantha	Twig	May, 1931	Ithaca, New York
507	Crataegus crus-galii	Twig	July, 1931	Ithaca, New York
"Noble"	Pyrus communis Bartlett	Root	Jan. 4, 1932	Walnut Grove, Calif.
SC	Pyrus communis	Twig	June, 1925	South Carolina

[a] In this paper the word strain is used as synonymous with the isolate.

[b] This culture, isolated by E. F. Smith, was received from the American Type Culture Collection Bureau under the number 493.

hundred individuals were used to compute the mean size of the cells of each isolate. In the present work only the length of the organism was considered. Measurements were made by the use of the Spencer filar micrometer.

According to W. L. Trotzky (52), who studied variation and significance of length in *Bacillus typhosus* and *B. dysenteriae*, the length of bacteria may be considered as an individual and constant characteristic of each strain. Strains that were nearly identical in morphological, biological, and serological properties very often were characterized by the fact that the length of the bacterial cells seemed to be very constant and to be hereditarily transmitted.

Using the method outlined above, the writer obtained the following data on length of the different isolates. Isolate 501—0.93 μ; isolate 64—1.04 μ; isolate 507—1.09 μ; isolate 55—1.30 μ; isolate "Noble"—1.30 μ; isolate 57—1.38 μ; isolate 500–B—1.40 μ; isolate 50—1.50 μ; isolate SC—1.50 μ; isolate 77S—1.71 μ; isolate R (rough form of culture)—1.63 μ. To find how significant these measurements were, isolate 64 was mixed with isolates 50 and 77S and, after 3 successive passages at 24-hour intervals in nutrient broth of pH 6.9, the mixed culture was plated out on nutrient agar. After 48 hours of incubation at 28° C. several transfers of single colonies were made on slants of nutrient agar and kept for 2 weeks at room tempera-

ture after a preliminary incubation of 24 hours at 28° C. Subsequently, the cultures were transferred to slants of nutrient agar and 24 hours later smears were made as in the first experiment. The difference in length was so evident that there was no doubt that the cultures represented different isolates. The arithmetical means obtained for cultures used in this last experiment were: 1.07 μ, 1.47 μ, 1.68 μ. The mean in each case represented the average of 200 individuals for each culture.

It is interesting to note that the magnitude of length was in every case greater for isolates that proved to be slightly or very slightly virulent. There exists no correlation between the host from which the isolate came and the length of the organism.

Size and Form of Colonies. Ordinary nutrient agar did not depict strikingly the difference in size and shape of colonies between different isolates of *Erwinia amylovora.* However, if to this agar there was added 5 per cent commercial sucrose, characteristic features of differential value could be easily observed (Table 2).

TABLE 2.—*Colony characteristics of ten isolates studied*

Isolate	Description of colonies on 5 per cent sucrose nutrient beef-extract agar
50	Very slow grower. Colonies visible after 48 hours' incubation at 28° C. Colonies small, convex, entire, compact, granular.
77S SC	Grow very slowly. Colonies visible after 48 hours' incubation at 28° C. Colonies small, lobate.
55 57 64 501 507	Colonies small after 24 hours. After 48 hours' incubation at 28° C. Colonies large, round, shiny, moist with the edges thin and very transparent. The growth has tendency to spread so that in a few days there are very many coalescent colonies.
Noble	Slightly smaller than 55.
500–B	Like 55 but erose.

All isolates were grown also in 10 per cent sucrose nutrient agar. The characteristics of the colonies approached those on 5 per cent sucrose nutrient agar, but, after 5 to 7 days, there appeared a peculiar translucent outgrowth from the margin of the colony. This growth was of jelly-like consistency and, upon direct microscopic examination, was found to contain living bacilli. When small portions of this growth were transferred to nutrient beef broth of pH 6.9, there could be observed no growth in many tubes thus inoculated. There was a striking slowness in the appearance of colonies on plates and, again, some plates showed no growth of any kind, even after 20 days of incubation. Such colonies, on a nutrient agar plate,

were very small, translucent, slightly convex, and white and shiny by reflected light. The growth dies out on the plate within a week. This phenomenon was rather common for each isolate studied on 10 per cent sucrose nutrient agar. The pathogenicity and other properties of this type of *Erwinia amylovora* will be discussed later.

It already has been pointed out that the different isolates showed different rates of growth on the same media. To obtain an idea of the relative colony size of each of the isolates, they were inoculated into nutrient broth of pH 6.9 and transferred daily for 3 consecutive days. After this time plates were poured using nutrient beef-extract agar of pH 6.9 and incubated for 48 hours when the measurements of colonies were made. Each value for the size of colony of an isolate is represented in table 3 by an arithmetical mean of 250 individual colonies.

TABLE 3.—*Arithmetical means of colony size of 10 isolates of Erwinia amylovora*

Isolate No.	64	Noble	507	SC	57	50	501	55	550–B	77S
Mean colony diameter in microns ...	1073.6	644.2	568.5	550.5	532.8	516.5	494.6	402.5	389.3	355.2

It will be seen that there is a tendency for more pathogenic isolates to form larger colonies, excepting the large-size rough colonies to be mentioned later.

Eosin-methylene blue and Endo media were tried for differentiation of strains of *Erwinia amylovora*. The Difco brand of these media was used. Eosine-methylene blue medium shows rather distinctly the difference between strongly and weakly pathogenic strains. The less virulent strains always show a metallic lustre, absent in strongly virulent cultures. The slanted medium appears to be better for practical purposes, as it gives more rapid growth and is better for reading results. On Endo agar plates the growth for all the isolates studied was alike. No important diagnostic value can be assigned to this medium because of the sensitivity of the medium to light and consequent difficulty in reading the colors.

Lithium chloride agar[2] gave a rather sharp differentiation of weak and strong isolates. In this medium isolates 50, 77S, and SC appeared as very flat, translucent, spreading; while the rest of the isolates were raised, not spreading, and more or less opaque. No involution forms were observed on this medium.

[2] $MgSO_4$, 3 gms; K_2HPO_4, 2 gms.; $(NH_4)_2PO_4$, 6 gms.; lithium chloride, 5 gms.; sucrose, 20 gms.; distilled H_2O, 1000 cc., agar 20 gms. Medium adjusted to pH 6.9.

Motility. Stained by the Casares-Gil method, the organism was found to be motile by peritrichiate flagella, as shown by earlier workers (9). No difference in motility was observed between the different isolates. Arthur's (4) observations as to the organism being more sluggish in old cankers than in very recent infections could not be substantiated. The organism seemed to be equally motile, irrespective of the stage of the infection.

PHYSIOLOGY

To check previous work on cultural characteristics of *Erwinia amylovora* and to study the extent of cultural variability, the 10 isolates were used.

Temperature Relations. The 10 isolates were grown in nutrient broth and in a synthetic medium.[3] In each case pH was adjusted to 6.9 with $\frac{n}{10}$ NaOH. Inoculation was made with a 2-mm. loop from the growth on nutrient agar slants incubated at 28° C. for 48 hours. The amount of medium in each test tube was 10 cc. Readings were taken after the first 24 hours. The temperature at which cloudiness of broth was the heaviest was considered to be the optimum. The experiment was repeated 3 times, similar data being obtained in all cases. It was evident that the optimum temperature of growth for most of the isolates studied is 28° C. The isolates that were designated as weakly pathogenic are slower in growth than those that are strongly virulent. It is important to note that some isolates grow fairly well at low temperatures, such as 15° C. and 19° C.

To determine the temperature at which *Erwinia amylovora* completely terminates growth and multiplication, the same tubes were used as for the determination of optimum temperature. Tubes were kept longer at low and high temperatures. After a week's incubation it was found that at 3° C. there was no perceptible growth in any tube; at 8° isolates 64, 501, and 57 showed fairly good growth; at 12° all the tubes were cloudy; at 31° and 34° all the tubes showed fair growth. No growth was observed in tubes incubated in 37° and 40° C. chambers. Isolates that showed no growth at 3° and 8° were plated out and the plates with original tubes placed at 28° C. Tubes were cloudy in 24 hours and colonies were visible on plates after 36 hours. The organism may remain viable at 3° C. in nutrient broth as long as 3 months without any transfer to fresh medium.

Death Point. The upper thermal death point for *Erwinia amylovora* has been placed at 43.7° to 49° C. Two thermal death-point tests were made, employing quadruplicate cultures in each trial. Trials were made at the following temperatures: 45.1°, 48.3°, and 49.5°. The trials were

[3] The ingredients of the nutrient broth were as follows: Peptone (Difco) 10 gms.; beef extract (Liebig), 3 gms.; NaCl, 5 gms.; distilled water, 100 cc. The composition of the synthetic medium was: $MgSO_4$, 0.3 gms.; $(NH_4)_2PO_4$, 6 gms.; KCl, 2 gms.; glucose, 20 gms.; distilled water, 1000 cc.

made for isolates 64, 50, SC, 500–B, and R, selections being made on the basis of relative pathogenicity of these isolates to pear. The isolates 50, SC and R, failed to grow in broth and agar cultures after 10 minutes' heating at 48.3°, but grew well after the same exposure at a temperature of 45.1°. The isolates 64, SC and 500–B failed to grow in broth and agar cultures after 10 minutes' heating, at 49.5°, but grew well after the same exposure at a temperature of 48.3°. Thus the upper thermal death point for isolates 50, SC, and R is between 45.1° and 48.3°, and for isolates 64 and 500–B, between 48.3° and 49.5° C.

Hydrogen-ion Concentration. D. H. Jones (29) reported a neutral reaction as the optimum for *Erwinia amylovora,* while V. B. Stewart (46) found the optimum reaction between 8 and 16 on Fuller's scale, which, converted into pH values by means of the formula of Quirk and Fawcett (42), would give pH 7.4 and pH 6.8, respectively.

To study this point further, nutrient broth and liquid synthetic media[4] were prepared. The pH for each lot of medium ranged from 3 to 10 with intervals of 0.2 of a unit and was checked by both colorimetric and quinhydrone methods. Each isolate was inoculated into 3 tubes of each pH and after 24 hours the reading was taken. It was found that the optimum pH at 28° C. for most isolates is 6.8 or slightly above. Moreover, there was a variation in acid tolerance among the isolates. Isolate 50 is able to grow at pH as low as 4.0, and isolate 64 at pH 4.4, while isolate 507 has the narrowest pH range of all. Most of the isolates grew from pH 4.8 to pH 8.8. These data agree fairly well with those of Pierstorff (41). On the alkaline side all the isolates behaved alike and this is in agreement with Howard's (27) findings. It must be pointed out that the growth in synthetic medium at low hydrogen-ion concentrations was not very prompt. The low pH at which the growth of *Erwinia amylovora* may take place helps to explain why the organism thrives in green pear fruits which have pH range of 4.2–5.36 (40).

Desiccation. The influence of such a physical factor as desiccation on the life of *Erwinia amylovora* is no doubt of some importance. L. R. Jones (30) could not recover the organism from a cover glass after 76 hours' drying at room temperatures. D. H. Jones (29) and V. B. Stewart (46) recovered the organism after 6 and 9 days, respectively, of drying on cover glasses at room temperature. Hotson (26) found the organism alive on branches about 4 cm. in diameter, which were dried in the laboratory 39 days after removal from the tree. In exudate from pear fruit, black and hard, the organism was alive after 13 days of direct exposure to sunlight and after 14 days with fruit of Jonathan apple. Thomas (48) has studied

[4] The synthetic medium was composed of $MgSO_4$ 0.3 gms.; K_2HPO_4 2 gms., NaCl 3 gms., asparagin 4 gms., dextrose 10 gms., and distilled water 1000 cc.

the desiccation of *Erw. amylovora* on the surface of the honey frame and on the surface of the comb. He found the organism still alive after 20 days on the frame, and after 50 days on the surface of the comb. Parker,[5] investigating the longevity of *Erw. amylovora* on combs of the beehive, states that the organism "was not recovered from artificially infected combs kept inside the hive through the winter. In the laboratory it survived a relatively short time (less than 17 days) at the higher temperatures and higher humidity reasonably near the conditions obtaining in the hive itself." It is evident that under certain favorable conditions *Erw. amylovora* can survive in a dry state for a very long time. Pierstorff (41) found organisms still viable in the dried bacterial exudate (ooze) that had been kept in the laboratory in a vial for 2 years. The writer was able to isolate viable bacteria from bacterial exudate kept in a vial in a dry laboratory closet for 2 years and 10 months.

To ascertain if different isolates of *Erwinia amylovora* are alike in their reaction to desiccation, 2 different experiments were performed. There were only 4 isolates used in these tests, namely, 64, 500–B, SC, and R. In the first experiment the isolates were grown on beef-extract nutrient agar for 24 hours; suspensions of the growth then were made in 0.85 per cent NaCl solution. Using a 2 mm. platinum loop, one drop of each suspension was smeared over sterile cover glasses so as to form a very thin film. The cover glasses were placed on the vaselined inner surface of the lid of a moist chamber. Different degrees of dryness from 10 to 100 per cent were provided by the use of the Lesage (34) method. Exposure over the surface of water was considered as at 100 per cent relative humidity. One series was included for laboratory air. A control for vaseline also was included. The exposure time for this experiment was as follows: 10, 20, 40, 55, and 60 minutes; 2, 4, 8, 10, 12, 16, 18, 20, 24, and 36 hours. The organisms survived under the conditions of the experiment from 18 to 36 hours. The degree of humidity seemed to be of no significance, as far as the longevity of *Erw. amylovora* is concerned in this experiment. However, culture SC was killed in a shorter time (18 to 20 hours) at all humidities than the rest of the cultures (24 to 36 hours), while culture R was the most resistant culture in the experiment. This property of withstanding adverse conditions by some isolates may perhaps help to explain the awakening of some old and dried up cankers, which, even by some experienced blight men, may be pronounced as extinct.

In the second experiment, nutrient broth, to which strips of gauze 3 inches long and $\frac{1}{2}$ inch wide were added, was sterilized and inoculated with the same isolates as in experiment one. After 48 hours the gauze strips were

[5] Parker, K. G. The fire-blight disease (*Erwinia amylovora* (Burr.) Comm. S.A.B.): Overwintering, dissemination and control. (In press.)

removed and suspended in large sterile test tubes kept at room temperature. Pieces of these strips were cut off and cultured from time to time. This experiment showed that isolates 64 and 500–B were not viable after 6 days; isolate SC could not be recovered after 4 days, and isolate R was killed after 10 days.

Both experiments were repeated and in each case the same results were obtained. Thus, it is safe to conclude that different degrees of susceptibility to dryness exist among isolates of *Erwinia amylovora* and that the rough type is characterized by greater resistance to adverse dry conditions.

CULTURAL CHARACTERS

Nutrient Beef Broth. Growth is prompt after 24 hours' incubation at 28° C. Isolates 50, SC, and 77S grow very slowly at pH 6.9, but grow promptly at pH 7 to 7.2. An occasional pellicle in the form of a ring was observed in isolate 507.

Potato-dextrose Broth (pH 6.9). Prompt growth after 24 hours' incubation at 28° C. Isolates 50, 77S, and SC grew slowly the first twenty-four hours. Slight sediment was formed by isolates 64, 55, 57, 500–B, 501, 507, and "Noble", and more sediment formed by 50, 77S and SC.

Beef-extract-Peptone Agar. Streaks on nutrient agar slants of different hydrogen-ion concentration did not reveal any striking characters. Usually the colony is white, glistening, moist, filiform, butyrous.

The use of 5 per cent sucrose nutrient agar is very valuable in bringing out certain differences between isolates. All isolates grow very luxuriantly on this medium, and differences are most striking after 3 days' incubation at 28° C. On this medium some isolates were characterized by white, translucent and spreading growth. The mass of organisms flows down and collects at the bottom of the slant in a very short time, leaving on the slant a thin translucent film. To this type belonged isolates 64, 501, and 55. Some isolates were characterized by grayish, heavy growth along the streak, raised, echinulate, coarsely granular by transmitted light, butyrous. To this type belonged isolates 500–B, 57 and "Noble". Some isolates were characterized by white, not spreading, beaded growth, with very abundant islands, and butyrous. To this type belonged isolates SC and 507. Other cultures possessed grayish, not spreading, filiform, slightly raised growth, less shiny than in group one, and finely granular by transmitted light. To this type belonged isolates 50, and 77S.

Gelatin Liquefaction. Gelatin-liquefaction trials were made, employing Frazier's method (16) for the detection of proteolysis. Also, a synthetic medium of the following composition was used: $MgSO_4$, 0.3 gms.; $(NH_4)_2PO_4$, 6.0 gms.; KCl, 2.0 gms.; glucose, 10 gms.; gelatin, 200 gms.; distilled water, 1000 cc. The reaction of this medium was pH 7.0 after

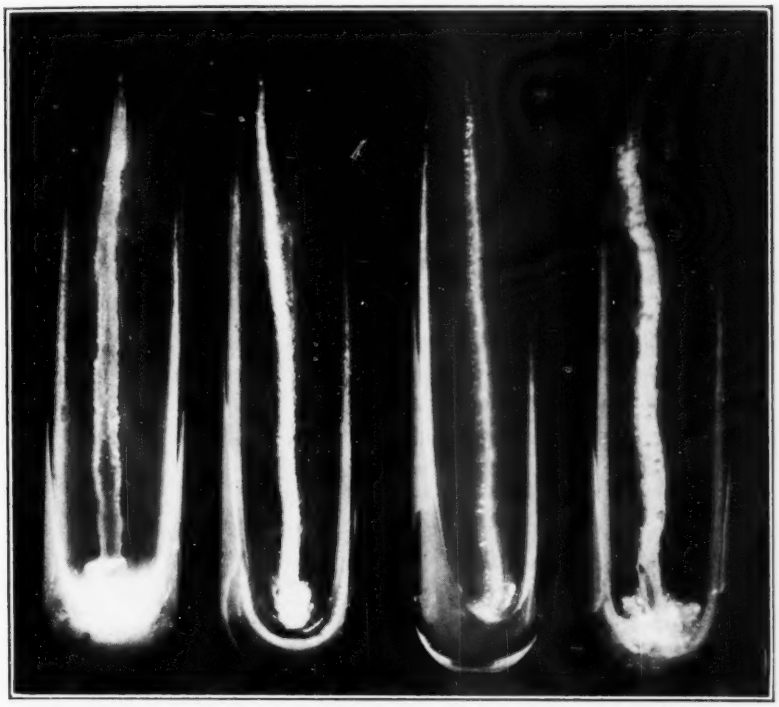

FIG. 1. Isolates of *Erwinia amylovora* on slants of a 5 per cent potato-sucrose-peptone agar. The isolate numbers from left to right are as follows: 64, 50, 500–B, and SC.

sterilization in the autoclave. All of the isolates studied, except 507 and 57, gave positive tests. Gelatin-liquefaction tests indicate proteolytic activities of *Erwinia amylovora* from none to very strong. Pathogenically weak strains show proteolytic activity far greater than that of the most pathogenic strains of the test.

Indol Production. None of the isolates of *Erwinia amylovora* produced indol after 20 days in Dunham's solution.

Nitrate Reduction. The organisms were grown in nitrate broth for two weeks and tests were made for the presence of nitrites. There was no reduction of nitrates to nitrites by any of the isolates. However, reduction of nitrates to ammonia was found for isolates 64, 55, 57, 500–B, 501, 507, and "Noble", and not for cultures 50, SC, and 77S.

Action on Milk. Skimmed milk plus 1 per cent of litmus indicator was distributed in 8 cc. amounts in test tubes, sterilized in the Arnold for 3 consecutive days and then inoculated. For each isolate 3 test tubes were inoculated and incubated at 28° C. for various periods of time. From the results obtained it is evident that the final principal action of the isolates

on milk is gradual digestion of the casein. No striking differences between highly pathogenic and weak strains could be observed, though the weaker strains seemed to decolorize the litmus indicator in a shorter time than the stronger strains.

Action on Starch. There was no diastatic action on starch (iodine test) shown by any of the isolates of *Erwinia amylovora.*

Ammonia Production. All isolates except 50, 77S, and SC gave a strong positive test for ammonia in nutrient broth after 10 days, using the method of Hansen (21) and Makris (36). Isolates 50, 77S, and SC gave a very slight positive test for NH_3.

Acetyl-methyl-carbinol Production. The inability of the organism to produce acetyl-methyl-carbinol in peptone-sucrose broth was shown by the Voges-Proskauer (54) test.

Carbon and Nitrogen Metabolism. Sugars, Glucosides, Organic acids, and Alcohols.—The organisms were grown in a synthetic medium ($MgSO_4$, 0.3 gms.; K_2HPO_4, 2.0 gms.; $(NH_4)_2PO_4$, 6.0 gms.; H_2O, 1000 cc.) to which 1 per cent of various carbohydrates, alcohols, and glucosides was added. In order to test the reaction of the medium during bacterial growth, 0.5 cc. of a 1 per cent alcoholic solution of brom cresol purple and 0.4 cc. of a 1 per cent solution of cresol red was added to each liter of medium (11). The medium was sterilized by filtering through a Berkfeld V candle. A small tube with a closed end was inverted in each tube of medium and used in place of the usual fermentation tube to determine the presence of gas and also whether or not the organism would grow anaerobically. The cultures were inoculated in triplicate from a 24-hour-old agar slant and examined at regular intervals of 48 hours.

In table 4 there are presented data on the growth and reactions of the different isolates in sugars and acids. In all sugars utilized by the isolates there was production of acid but no gas. All the isolates used inulin with the production of alkali and dextrin with the production of acid. Of the glucosides, arbutin, phloridzin, salicin, and amygdalin were fermented with production of acid.

Amino Acids. Table 4 shows the behavior of the different isolates of *Erwinia amylovora* in synthetic liquid media in which the source of nitrogen was substituted by the corresponding amino acid. Another experiment was made in which the source of both carbon and nitrogen was amino acid. Because the results in both cases were the same, the data are presented together. Of all amino acids tried in the experiments, alanine, leucine, and proline were used by some isolates, while asparagin, which is the amide of aspartic acid, is utilized by all the cultures tested and serves as a source of both nitrogen and carbon.

Relation of Asparagin to Infection. It is a general experience that green

TABLE 4.—*Fermentation of sugars, alcohols, glucosides, organic acids and other compounds*

	Not fermented by any isolate	Fermented by all isolates	Fermented by the isolates listed
Carbohydrates	Xylose Rhamnose Starch	With production of acid: Arabinose, mannose, glucose, fructose, maltose, cellobiose, sucrose, raffinose, dextrin. With production of alkali: Inulin	With production of acid: *Galactose* 64, 50, 55, 57, 77S, 500–B, 501; *Lactose* SC, 507, "Noble", 64, 50, 77S, 501.
Glucosides		With production of acid: Arbutin, phloridzin, salicin, amygdalin.	
Alcohols	Dulcitol		With production of acid: *Manitol* 64, 50, 55, 77S, 500–B; *Glycerol* 50, 55, 77S, SC, 500–B, 501, 507, "Noble".
Amino-acids	Glycine, valine, isoleucine, glutamic acid, cystine, cysteine, tyrosine, tryptophane.	With production of alkali: Asparagin	With production of alkali: *Alanine* 77S, SC; *Leucine* 50, 77S, SC, 501, 507, "Noble"; *Proline* 55, 77S, SC, 500–B, 501, "Noble".
Organic acids	Ammonium benzoate, ammonium oxalate, maleic acid, malonic acid, sodium benzoate, sodium salicylate, tartaric acid, valeric acid.	With production of alkali: Ammonium citrate, citric acid, hippuric acid, malic acid, sarcosine, sodium citrate.	With production of alkali: *Succinic a.* 500–B, 501, 507, "Noble"; *Ammonium lactate* 64, 50, 55, 77S, SC, 500–B, 507, "Noble".
Miscellaneous	Melanamide Urea		With production of alkali: *Edestin* 64, 57, 77S, SC, 501, 507, "Noble".

pear fruits are especially susceptible to "fire-blight". According to analyses at hand (56) the pear fruit contains from 0.42 to 0.52 per cent of asparagin, dry weight. The mature pear fruit contains less than 0.1 per cent of asparagin, dry weight. Disregarding pH of the fruit juice, the value for which remains about the same, according to Overholser (40) and Buxton and Darbishire (10), can the difficulty of obtaining infection on ripe pear fruits be correlated with very low asparagin content of the pear juice?

To gather some idea as to how significant asparagin may be in initiating or spreading infection in susceptible and resistant plants, a series of experiments was made. It is well known that fire-blight infection cannot readily be produced by artificial inoculations in a dormant susceptible plant and only occasionally during the growing season in shoots that have ceased termi-

nal growth. It seemed that if infection could be started in such plants after injecting asparagin, the conclusion as to asparagin having played some rôle could be justified.

In one of the experiments a Bartlett pear tree, growing in a balanced solution, and in a very dormant condition, was inoculated with isolate 64 to which asparagin was added just before inoculation; control shoots were inoculated with isolate 64 without asparagin. At the end of 2 weeks the shoots inoculated with isolate 64 plus asparagin were blighting fairly well, while those of the control showed no infection.

In another experiment there were selected 2 2-year-old Winter Nelis pear seedlings, which had made no growth for a considerable time, and, though they had some leaves on them, the buds were hard and mature. Into one of these seedlings was injected 50 cc. of 1 per cent asparagin solution in water, while another plant was given by the same method 50 cc. of distilled water. Plants were inoculated in dormant buds with a 24-hour-old culture and placed in a warm greenhouse, where they were watered daily. After 3 days the seedling injected with asparagin showed a sizeable drop of ooze, while the control plant was free of any signs of infection, even after 2 weeks. The plant treated with asparagin showed a canker 2 weeks after inoculation.

In still another experiment a very resistant plant of *Cotoncaster frigida*, which had not become infected by inoculation with a virulent culture of *Erwinia amylovora* in a number of trials, was given, through injection, 50 cc. of 1 per cent asparagin and afterwards inoculated with a virulent strain of *Erw. amylovora*. Blight was visible 24 hours after inoculation.

In the course of studies of variability of different isolates of *Erwinia amylovora*, it was interesting to know to what extent asparagin is tolerated by the organism. For this purpose a synthetic medium similar to the one described on page 6 was used. To this medium varying amounts of asparagin (Pfanstiel) were added. In concentrations higher than 6 per cent, asparagin formed crystals in the medium. The experimental data showed that the isolates tolerate as high concentrations of asparagin as 6 per cent and thrive very well, utilizing asparagin as a source of both nitrogen and carbon. The weaker strains grew with their usual slowness.

Relation of Sugar Concentration to Growth of the Fire-blight Organism. Considering the readiness with which the organisms grow in glucose and sucrose sugars, it was interesting to know what concentration of each of these sugars would inhibit growth. For this purpose the isolates were studied in a liquid synthetic medium to which varying amounts of glucose and sucrose were added. The medium was sterilized by filtration through Berkefeld V filter candles and tubes were allowed to incubate for a week at 28° C. before inoculations were made. The data indicate the ability of *Erwinia amylovora* to tolerate relatively high concentrations of sucrose and glucose: All isolates

grow in 50 per cent sucrose, isolates SC, 501, and 77S failed to grow in 55 per cent sucrose, isolates 507, 50 and "Noble" grow in 60 per cent sucrose. In glucose all isolates but "Noble" tolerate 14 per cent; isolates 64, 500-B, and 77S tolerate 16 per cent; isolate 507 grows in 20 per cent but not in 22 per cent; isolate 57 grows in 25 per cent but not in 26 per cent, and isolate 50 grows in 28 per cent. Isolate "Noble" tolerates 10 per cent but not 12 per cent.

The tubes that showed no growth in the above tests were cultured in the following manner. One cubic centimeter of the content of the test tube was transferred into sterile nutrient broth and another cubic centimeter portion was smeared on the surface of agar in Petri dishes. This was incubated for a week. In no case was growth obtained.

VIRULENCE

Arthur (4) was one of the first investigators of fire blight to attempt to "learn why some varieties suffer more from the disease than others". He made a number of inoculations and cross inoculations into pear, apple, quince, etc. Arthur's work is very suggestive of the existence of differences in the organisms used. Jackson (28) isolated the organism of the fire-blight disease from prune and found it to be more virulent than that from pear. Sackett (43) observed differences in incubation periods of organisms isolated from different plants. Pierstorff (41), employing twelve different isolates of *Erwinia amylovora,* made an attempt to find physiological strains. He was able to observe in some cases distinct differences in the percentage of infected twigs and in the average length of twig blighted.

The writer undertook a comparison of variability in virulence of the 10 isolates used elsewhere in this work. The term virulent as employed in this work designates the power to rapidly spread within the tissues producing death of invaded parts. To judge the relative virulence of isolates the percentage of blighted shoots and average length of twig blighted are taken as criteria. Many inoculations were made in the greenhouse using potted plants. A great number of apple and pear seedling plants were used in inoculations at the University experimental plots in Berkeley and San Jose, California. In some cases the cultures used were single-cell cultures. Inoculations in all cases were made into succulent tender tips with the inoculating needle, using 48-hour-old agar cultures. Readings on infection were made at the end of the third week after inoculation.

Table 5 represents the data on inoculations into seedlings of the Beurre Hardy variety of pear and *Pyrus malus.* It is evident that isolates 50, 77S and SC behave similarly on the same host and are physiologically distinct.

From 53 to 230 inoculations (usually 100 to 150) were made for each isolate on each of the following: *Cotoneaster frigida, C. pannosa, Photinia*

TABLE 5.—*Infection produced by different isolates on seedlings of Beurre Hardy pear in the field at San Jose, May–July, 1932, and on seedlings of Pyrus malus in Berkeley, July–August, 1932*

Isolate	Beurre Hardy pear				*Pyrus malus:* 100 inoculations per isolate	
	First experiment: 500 inoculations per isolate		Second experiment: 500 inoculations per isolate			
	Per cent infected	Average length of twig blighted (in.)	Per cent infected	Average length of twig blighted (in.)	Per cent infected	Average length of twig blighted (in.)
64	98	4.31	92.0	3.90	100	7.09
50	5.2	2.10	4.4	1.78	0	0
55	91.4	5.02	96.0	3.82	90	7.55
57	61.0	4.42	80.0	4.46	71	3.58
500–B	86.6	5.32	92.8	5.45	83	5.91
501	87.6	4.94	78.8	3.27	92	6.28
507	89.2	5.42	96.4	4.96	100	5.52
77S	11.0	3.26	10.0	3.47	6	1.46
SC	29.6	3.14	26.8	3.25	5	2.00
"Noble"	98.6	4.65	86.0	4.70	100	5.54

arbutifolia, Pyracantha angustifolia, P. coccinea lalandi, P. gibbsii younnanensis. The results were similar in the main to those with pear and apple but with certain deviations. With *C. pannosa* and *P. arbutifolia,* which are highly susceptible under the conditions of these tests, isolate SC with the former and isolates 50 and SC with the latter were distinctly less virulent than the remaining isolates. On the other hand, with the comparatively resistant *P. gibbsii younnanensis,* isolate 57 as well as 50, 77S and SC produced no infection while the remaining six isolates blighted from 14 to 45 per cent of the inoculated shoots.

The question was raised as to whether it would be possible to explain the peculiar behavior of isolates 50, 77S, and SC on the basis of prolonged culturing on artificial media. For this purpose some of the recently isolated isolates of *Erwinia amylovora* were used to make inoculations along with certain of the ten original isolates. It was found (table 6) that recently obtained isolates 2150 and 50[1] were as weak as isolates 50, 77S, and SC. On the other hand, such isolates as 64 and 507 which had been grown in the laboratory for about 2 years were among the most virulent and the former is still being used as a standard of high virulence after 4 years in culture.

These results constitute evidence of distinct physiological entities, and while prolonged culturing may account in part for the low virulence of some isolates, the close similarity of cultures 50 and 50[1] (table 6) taken from the

TABLE 6.—*Infection produced by old and new isolates of Erw. amylovora, June to August, 1933*

Isolate	Per cent infected		Isolate	Per cent apple infected: 100 inoculations per culture
	Pear: 250 inoculations	Apple: 100 inoculations		
93[b]	75	74	64	100
95[c]	68	76	50	0
2120[d]	30	96	501[a]	0
2150[e]	2	0	507	86
2115[f]	64	84	778	8

[a] Isolate 50—From same host as 50, but isolated May, 1933.
[b] " 93—From *Cotoneaster pannosa*, Berkeley, June, 1932.
[c] " 95—*C. salicifolia* " " "
[d] " 2120—Forelle pear " May, 1933.
[e] " 2150—*Pyracantha gibbsii younnanensis*, Berkeley, March, 1933.
[f] " 2115—Spitzenberg apple, Sebastopol, June, 1933.
For source of other isolates see table 1.

same suscept and locality at widely different dates, indicates that weakly virulent strains may arise in nature and persist there for rather long periods.

ORIGIN AND REVERSION OF VARIANTS

Dissociation in Bacteria. Animal Pathogens. In recent years the morphological variation of microorganisms, especially pathogenic bacteria, has been given considerable attention. The knowledge of this variability has practical significance in the diagnosis of a particular microorganism as well as in the other aspects of practical bacteriology, as for instance, serotherapy. The term dissociation in its more limited sense implies the segregation of two quite distinct bacterial forms which are always present in the parent culture and is associated especially with the idea of regular cycles through which bacteria pass in their life history. An excellent treatment of the whole subject of microbic dissociation is found in Hadley's (20) classical monograph on variation and it is deemed unnecessary to go into a detailed review of the subject at this time.

In 1921, Arkwright (2, 3) described two different variants in the dysentery and Salmonella groups of bacteria, namely, smooth ("S") form and rough ("R") form. Since that time the dissociation phenomenon has been observed and reported for many important bacterial pathogens of man and animals.

Plant Pathogens. It is only recently that the phenomenon of microbic dissociation has aroused the interest of phytobacteriologists. Though the

literature on the dissociation in plant pathogenic bacteria is meager, there is enough information to indicate that the phenomenon takes the same general course as with the animal pathogens. Miss Hedges (22) in 1924 and Sharp (44) in 1927 noted and illustrated the rough colonies of *Phytomonas phaseoli sojense,* Hedges. Gardner and Kendrick (17) described and presented a photograph of aberrant (rough) colonies of *Phytomonas vignae,* G. & K., and Link and Hull (35) in 1927 obtained rough strains of *Phyt. citri, Phyt. medicaginis,* var. *phaseolicola,* and *Phyt. tumefaciens. Phytomonas beticola* was reported to undergo very ready dissociation on nutrient laboratory media and in the host by Brown (8), Elcock (15), and O'Neal (39). In 1931 Stoughton (47) called attention to variants in *Phyt. malvacearum.*

Plant pathogens undergo dissociation on solid media and rough types are pathogenic to the hosts, though in some cases to a very slight extent. In some instances the rough types give more pronounced symptoms than the S form. Elcock (15) noticed that the R form of *Phytomonas beticola* produced overgrowths larger than those from the S type.

Characters of S, R, and Other Types. The smooth (S) type forms smooth, round glistening, moist, dome-shape colonies with sharply defined limits. Examined under the microscope the colony appears to be finely granular. In bouillon this type produces uniform cloudiness without pellicle. In physiological solution (0.85 per cent NaCl) the S type produces diffused turbidity without floccules. The colonies of the R type have a very different external and internal appearance. They are generally larger than those of the S type, flat, dry, with irregular margins. In the center of the colony there often is a small knob and the surface is wrinkled. In bouillon this form produces abundant precipitate and pellicle. In physiological solution the R type produces flocculation. The bacteria of S type are motile, those of R type slightly motile or nonmotile.

Besides these two mentioned forms, bacteriologists distinguish another type of colony, intermediate between S and R, and designated by the letter O or sometimes by I.

Agencies Producing Variants. Variants can be produced by chemical or immunological means, as pointed out by Arkwright (2, 3), Hadley (20), Hoffstadt (25), Koser (31, 32), Kwaschnina (33), Müller (38), not to mention a score of others. Sometimes these variants appear spontaneously. Among the chief factors concerned in the causation of variants are aging of the culture, changes in temperature, food substances, other chemicals, physical state of medium, and specific immune sera.

Biological Variations in S and R and Intermediate Types. The dissociative processes bring about not only morphological differences in microorganisms but also produce biological variations in strains thus produced, such as difference in virulence and behavior in nutrient media, (19, 51,

57, 58). In some cases the rough type, which is avirulent when produced, may become highly virulent upon passage through the animal, as shown by Todd (50). In *Bacillus anthracis* there exists a peculiar situation in that the virulent type is usually rough, while the avirulent form is smooth. Eagles (13), in his study of two strains of haemolytic streptococci, found enhanced virulence in the rough variant of one and lack of virulence in that of the other, and concluded that colony appearance is not a reliable index of virulence. Andrews (1) found more serological specificity in a rough type of haemolytic streptococcus than in a smooth type.

Reversion of R into S. It generally is conceded that the R type of bacterial species is stable on common laboratory media, especially solid media. The reversion of the R type into the S is brought about by various methods, and there is no general rule laid down for all species of bacteria. The animal bacterial pathogens frequently are changed from R to S type by the passage of the R type through an experimental animal. There are several instances in which the R type was converted into S by use of agar and liquid media. Thus, Edwards (14) successfully reverted the rough type of *Shigella equirilis* into the smooth form by growing the organism on nutrient agar slants of acid reaction at temperatures above 37° C. Koser and Styron (32) reported the conversion of the rough type into smooth by daily or twice daily transfers of *Bacterium dysentery* in glucose broth. They state that "in some instances S colonies appeared as early as the fifth transfer." Both Sharp (44) and Elcock (15) found the reversion of R type into S occurred during passage through susceptible plants in case of *Phytomonas beticola* and *Phyt. phaseoli sojense,* respectively.

Dissociation in Erwinia amylovora. Observing the morphological, cultural, and pathogenetic variability of *Erw. amylovora,* the writer wondered if that could not be explained by the dissociation of the organism. Attempts were made to produce variants, and especially the rough form, by the methods known to bacteriologists. The following account gives the outcome of these studies.

Effect of Temperature on Dissociation of Erwinia amylovora. To see if the temperature factor is influential in bringing about changes in type of colony, nutrient beef broth of pH 6.9 and 7.2 was inoculated with the isolates of *Erw. amylovora* mentioned elsewhere in this work. The test tubes were placed in incubators at temperatures 3°, 8°, 12°, 15.5°, 19°, 21°, 25°, 28°, 31°, 35° 37°, and 40° C. Every 24 hours streaks from each test tube were made on nutrient 2 per cent agar plates which subsequently were incubated at 28° C. for 3 days, after which time the examination of the resulting growth was made.

This experiment showed that the dissociative process was operative at temperatures of 12° to 25° C., irrespective of pH. The first rough colonies

were observed after 20 days of incubation. The isolates 50, 77S, and SC were the first to show the complete dissociation into the R type (after 10 days), while the rest presented a mixture of R and S types after the 20th day.

The rough colonies were large, flat, wrinkled, and dull. They were firm when touched with the needle and when suspended in 0.85 per cent NaCl solution formed clumps. In all particulars they agreed with the descriptions of the rough type in other bacteria. When transferred to solid media the rough colonies appeared to be stable after numerous transplants. There was no tendency to revert when grown in common nutrient broth.

Effect of Aging on Dissociation. As has been mentioned above, the aging of the culture may result in the production of variants. Broth tubes and dilution plates and streaks on nutrient infusion-broth agar that were stored at room temperature for from 4 to 9 months were used in studying this point. All the cultures after prolonged storage showed an abundance of rough type colonies. Again, in tubes with isolates 50, 77S, and SC only rough colonies were present, while in the rest was a mixture of rough and smooth.

Continuous cultivation of the organisms on solid media, organic and inorganic, in no case leads to the appearance of dissociants. Occasionally, however, some isolates, especially SC and 77S, give rise to colonies with numerous minute papillae on the surface of the colony upon streaking on nutrient agar containing 0.5 per cent lithium chloride (Fig. 2, D). The dilution plates made from these papillae reverted to the normal type of colony.

Effect of Repeated Transfers in Bouillon. Another experiment was undertaken to ascertain if rapid transfer in liquid media could be used to cause dissociation. Nutrient broth of pH 6.9 was inoculated with the 10 isolates and transfers made into fresh broth every 18 hours. Tubes were inoculated at 28° C. Each tube, after taking a loopful for inoculation into fresh bouillon, was used for streaking agar plates. This procedure produced dissociants in some cultures. The dissociation was, however, very slow and incomplete. After thirty days isolates 50, 77S, and SC produced 10 per cent roughs and maintained it for 2 months. The experiment was then terminated. The other 7 cultures remained unchanged after 2 months.

Effect of Peptone and Meat Extract on Dissociation. It has been reported by Koser and Styron (32) that high concentrations of peptone were effective in bringing about microbic dissociation. With this in mind concentrations of Difco peptone from 1 to 20 per cent were tried. Dissociation took place only in one per cent peptone broth incubated at 25° C. for 20 days. Next, the concentrations of meat extract (Liebig's) were changed, the range used being from 0.3 per cent to 5 per cent. No special enhancement of dissociation was observed, the process taking place in the usual concentration of meat extract and in the same way as described above.

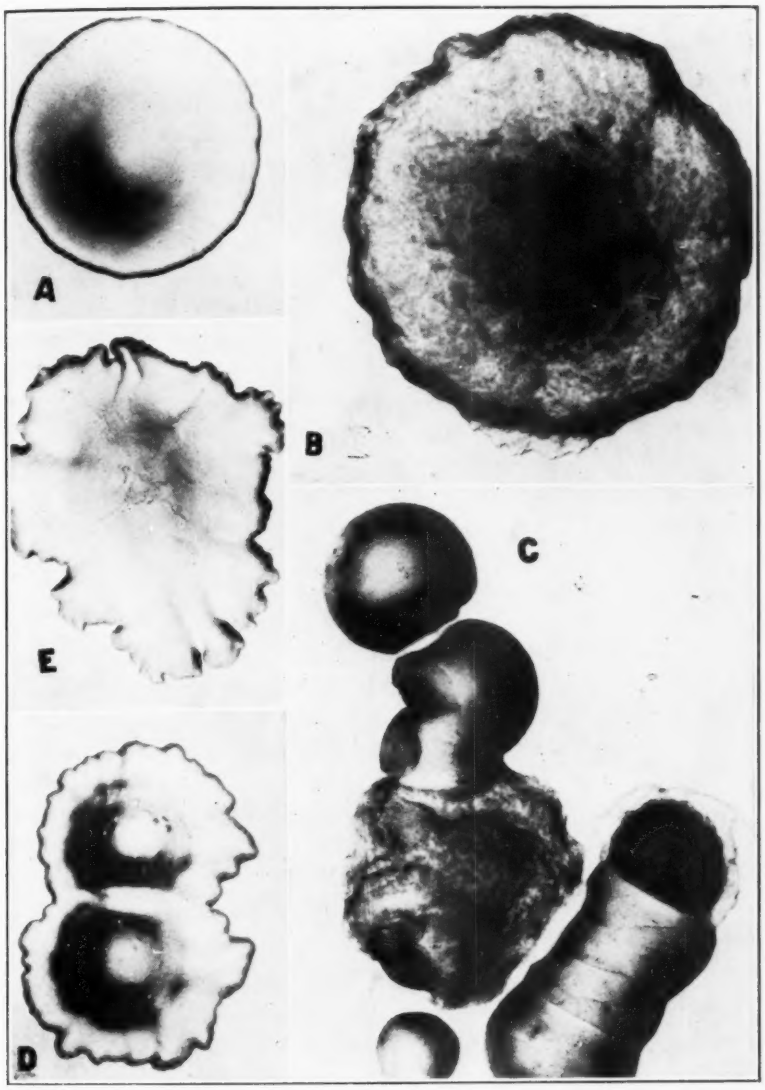

FIG. 2. A. Normal (S) colony of *Erwinia amylovora*. B. Rough type ("R") of *Erw. amylovora*. C. Mixture of rough and smooth colonies of isolate 64. Streak on potato-dextrose-peptone agar plate. D. Secondary daughter colonies on colony of isolate 507. Result of growth on lithium chloride medium. E. Erose colony of isolate 500–B.

The Effect of Sugars on Dissociation. Sucrose and glucose were not effective in bringing about dissociation; on the contrary they were found to be important in the reversion process.

Reversion of R to S. Since the rough type is stable and cannot be further

dissociated, it was important to gain some information as to conditions necessary to revert the R type into S. Some of the methods of medical bacteriologists were used. The rough type of *Erwinia amylovora* was grown in nutrient broth with pH range from 6 to 8 with intervals of half unit. The temperature range was from 29° to 37° with intervals of 2° C. No conversion of the rough type into smooth was obtained by these methods at the end of 1 month. Immune serum was without effect on the R type. However, when the rough culture was grown in 2 or 5 per cent sucrose nutrient broth, reversion was noticed after 4 to 6 daily transfers. The plating out of the rough culture at this time showed a mixture of R and S types. This conversion could not be observed in 10 per cent sucrose broth. The best glucose concentration for the reversion was found to be 1 per cent in nutrient broth. The effect of sugar on reversion is in agreement with findings of Koser and Styron (32) on dysentery bacteria. This phenomenon of reversion of *Erw. amylovora* from rough to smooth may be important in explaining the epiphytology of fire blight, as can be seen from the data to be presented later.

Occurrence of R Type in Natural and Artificial Infections. Does the rough type of *Erwinia amylovora* occur in nature? The answer to this question is, no doubt, significant in clarifying some confusion existing in our knowledge of fire blight. After the laboratory production of the rough type, closer attention was given to colonies appearing on the plate when culturing for the fire-blight organism. The technique used consisted in surface sterilizing the sample, crushing it in a mortar in sterile bouillon, and then making dilution plates, or streaking and smearing on agar plates with glass rods (23, 24).

It was found that the R type may be isolated from old infections, *i.e.*, the infections that are approaching their terminus, as for example old cankers on moderately susceptible trees. In one case a large canker on an apple root was received for isolation. This sample contained varied forms of *Erwinia amylovora,* among which the predominant types were R and O (intermediate). On several occasions old, cracking cankers on trunks of Bartlett pears yielded some rough and intermediate types along with the smooth type. Instances where the rough and smooth were found together in the ooze from cankers also are noted. In all these cases the rough type was very slightly pathogenic for succulent pear shoots and avirulent for shrubs like *Pyracantha angustifolia.* The rough type of *Erw. amylovora* was obtained from old twig infections produced by artificial inoculations into *P. angustifolia, P. gibsii younnanensis, Cotoneaster frigida* and *Pyracantha* (hybrid). Some samples of blight on *P. angustifolia* and *C. salicifolia* brought from Niles and San Jose, California, yielded a good crop of roughs.

Attenuation of Erwinia amylovora by 10 Per Cent Sucrose Broth. In the course of study of the influence of sugar on the behavior of *Erw. amylovora,*

another observation was made that is likely to be of interest in explaining the absence of disease or its very slow course in dry conditions. In studying the sugar tolerance of *Erw. amylovora* the writer noticed the attenuation of the virulent culture 64 when that culture was kept in sucrose synthetic medium of 10 per cent or more of sugar. Succulent shoots of pear seedlings inoculated with a one-week-old culture in 10 per cent sucrose showed a great delay in blighting compared with those inoculated with a culture from 2 per cent sucrose medium. This phenomenon was followed up and a medium containing 10 per cent sucrose was inoculated and daily transfers into fresh tubes of the same medium were made with simultaneous inoculations into soft green shoots of *Pyracantha angustifolia* (Table 7). It is interesting to note the decrease in virulence of almost 50 per cent as a result of the daily transfers in 10 per cent sucrose medium. The organism seemed to have lost some of the power to invade the tissues, but, once it gets into the tissues, it proceeds with the usual speed as indicated by the column on average length of twigs blighted.

Variants Other than S and R. Aside from the characteristic R type colonies, a number of variants of *Erwinia amylovora* was encountered during the course of this study. The intermediate type (0) was observed for many isolates and was very stable when kept on solid media as well as in liquid media.

Rhizoid and translucent types of colony were observed infrequently in some isolates. Isolates 507, SC, and 77S sometimes showed rhizoid colonies when plated out from 2- or 3-day-old agar slants. All isolates, when grown on 5 or 10 per cent sucrose nutrient agar, split off very translucent, small, thin, flat colonies that were characterized by a very slow growth on nutrient agar and in nutrient broth. This translucent type of colony had to be transferred at frequent intervals in order to keep the bacteria alive. In cultures 1 week old the bacteria were found dead.

Virulence of R and Other Variants. The rough type of *Erwinia amylovora* possesses very slight virulence to green fruits of susceptible varieties of pears and no virulence to the shrubs so far tested, *e.g., Cotoneaster dammeri radicans, C. frigida, C. pannosa, C. salicifolia, Photinia arbutifolia, Pyracantha angustifolia* and *P. gibbsii younnanensis.* Succulent shoots of apple seedlings were not susceptible to a rough strain of *Erw. amylovora.* The intermediate type of any isolate of *Erw. amylovora* studied was found to be pathogenic to all the above-mentioned hosts, though to a lesser extent than the smooth form.

At this point it is interesting to note that isolate 50, which behaved very much like the intermediate type, was dissociated into S and R types. The resulting R type was found to be avirulent for the shrubs tested and slightly virulent to pear fruits and succulent shoots. The S form of isolate 50 was

3 times as virulent as the parent culture. *Pyracantha angustifolia* was inoculated in the greenhouse with isolate 50 (parent culture) and the corresponding smooth form. Out of 150 inoculated shoots the S form blighted 126, or 84 per cent, while the parent culture blighted only 42, or 28 per cent. The average distance invaded by the smooth form was 1.32 inches; that of the parent culture 0.87 inches.

Isolate 50 produced also the translucent type of colony, which proved to be absolutely avirulent to pear, apple, and other rosaceous suscepts tested. This translucent type, however, may gradually be changed into a virulent one by repeated transfers into 2 per cent sucrose nutrient broth. Six daily transfers in that medium suffice to make an avirulent translucent culture as virulent as the mother culture. May not this be a factor in bringing about the epidemic of blossom blight when the concentration of sugars in nectaries falls to 2 or 3 per cent during moist weather conditions?

It already has been mentioned that sugars have a pronounced effect on the change of R type into smooth, and from less to greater pathogenicity. It must be said that this change is brought about gradually: the number of smooth-type colonies is gradually increased, and, with this, the virulence is enhanced. De Kruif (12) states that the virulence of a given culture is a function of the proportion of S bacteria present in it and is not due to a general rise or decline of the invasive capacity of each unit in the culture; while Zinsser (59) is of the opinion that organisms contain individuals of different types, and that differences in virulence depend upon the numerical proportions between highly virulent S organisms and the less virulent R types, different environments favoring the preponderance of one or the other. This idea is borne out by the following experiment performed to ascertain the pathogenicity of cultures when passed through 2 per cent sucrose nutrient broth. Isolate 50 (not dissociated) and strain R of isolate 64 produced by enforced dissociation were inoculated into 2 per cent sucrose nutrient broth of pH 6.9 and daily transfers were made into fresh tubes of the same medium. Every day after transfers were made, rapidly growing shoots of *Pyracantha angustifolia* were inoculated in the greenhouse. Table 7 indicates the increase in virulence gained by these isolates as a result of cultivation in sucrose nutrient broth of a low sugar concentration. The isolations of the organisms from blighted plants showed the presence of rough and smooth types up to the 8th passage, while after that only the S type was present in dilution plates made from the diseased tissues.

DISCUSSION

The wide fluctuation in the prevalence of fire blight from year to year is well known. The difference of opinion as to the causes of this fluctuation is perhaps equally wide. The principal controversy centers around the

TABLE 7.—*Infection produced on Pyracantha angustifolia in the greenhouse by organisms passed through different sugar concentrations. For each passage one hundred shoots were inoculated with each culture*

Passage	Isolate 50[a] (Not dissociated)		Isolate 64[b] (R type)		Isolate 64[c] (Smooth type)	
	Percentage of shoots blighted	Average length of twig blighted	Percentage of shoots blighted	Average length of twig blighted	Percentage of shoots blighted	Average length of twig blighted
1st	30	0.85	0	0	100	4.37
2nd	50	1.00	0	0	100	3.94
3rd	45	1.17	0	0	100	3.90
4th	75	1.15	65	0.43	95	3.66
5th	85	1.09	50	0.40	90	3.71
6th	90	1.23	67	0.83	90	3.68
7th	85	1.30	60	0.60	80	3.80
8th	85	1.30	60	0.88	70	3.75
9th	85	1.39	95	0.96	65	3.58
10th	95	2.01	95	1.58	53	3.50

[a] and [b] Passed through 2 per cent sucrose nutrient broth.

[c] Passed through 10 per cent sucrose in synthetic medium.

mode of dissemination of the organism, with less divergence in view as to the agencies that promote the establishment and development of infection. It has been shown that high atmospheric humidity favors the development of infections already established, and a few authors have taken the view that rain is the chief vector (18, 37, 53). Many investigators, on the other hand, are convinced that insects are the principal agents in dissemination (7, 6, 42, 55).

Some work recently reported from this laboratory (49) together with the matter presented in the foregoing pages may perhaps partly reconcile the divergent hypotheses as to dissemination, and may offer a more exact explanation of the factors governing the inception of infection and its subsequent rate and degree of development.

Dissociation of *Erwinia amylovora,* brought about by such agencies as adverse temperatures, age of the infection, change in abundance or kind of food, may well be expected to take place in nature, thus giving rise to variants of widely different virulence. This is shown to be the case in isolates from old infections of pear and apple and certain shrubs that yielded smooth (virulent), intermediate and rough (less virulent) types.

It was found further that continued culturing of the organism in media containing as high as 10 per cent sugar resulted in attenuation of the organism to the point of slight or no virulence (translucent type). Certain higher sugar concentrations within the range found in nectars, inhibited

growth altogether. On the other hand, repeated transfers in media of low sugar concentration were followed by restoration of the attenuated translucent type culture to the parent type and of rough and intermediate forms to the virulent smooth form. It has been shown by Beutler (6) and earlier workers, and verified in California by Vansell[6] and others (50), that the sugar concentration in the nectaries of blossoms varies widely in inverse relation to the atmospheric humidity, and the volume of nectar undergoes variations of similar magnitude in direct relation with humidity. Thus the nectar of pear may vary from 1 or 2 per cent total sugar in an essentially saturated atmosphere to at least 55 per cent in very dry air. It seems apparent then that rainy or humid weather may profoundly influence the course of fire blight, chiefly in an indirect way, by determining the volume and sugar concentration of the medium that the organism finds in the principal infection court (nectary), which in turn may not only favor the rapid increase of virulent forms of the bacteria but also change the weakly pathogenic forms to the virulent state. A complete hypothesis to account for the development of epiphytotics of blossom blight also would include favorable temperature and intervals of weather conducive to the flight of blossom-visiting insects.

SUMMARY

Ten different isolates of *Erwinia amylovora* representing 6 localities and 8 suscepts were studied.

Morphological studies show that *Erwinia amylovora* varies in size of the individual cells, and in size and form of the colony. Length of individual bacteria from strongly and moderately pathogenic isolates was found to vary from 0.93 μ to 1.40 μ, for weakly pathogenic ones from 1.50 μ to 1.71 μ. The length of bacterial cells appears to be a stable characteristic of the culture.

Marked variability of *Erwinia amylovora* in virulence was noted and correlated with morphological and some physiological characters.

Eosine-methylene-blue medium was found to differentiate between strongly and weakly pathogenic isolates in that the latter produce metallic lustre on slants of this medium.

Isolates were found to tolerate pH as low as 4.0 and as high as 8.8.

The upper thermal death point was determined to be between 45.1° and 48.3° C. for weaker isolates and between 48.3° and 49.5° C. for more pathogenic isolates.

The organisms survived on cover glasses for 24 to 36 hours with little apparent relation to humidity. In gauze strips the organisms lived from 4 to 10 days.

Cultural reactions of 10 isolates to sugars, alcohols, glucosides, amino acids, some proteins, fatty acids, and amides are reported. Variation was

[6] Vansell, G. H. Bee behavior as affecting pollination. (In press.)

noted among the isolates in their capacity to utilize certain of these chemicals.

Injections of asparagin into very resistant plants or into completely dormant plants seemed to promote infection upon subsequent inoculation.

Nine isolates grew in 50 per cent sucrose and one in 60 per cent sucrose. In glucose all isolates but one tolerated 14 per cent and one isolate grew in 28 per cent.

Dissociation of *Erwinia amylovora* was observed and studied. Aging of the culture invariably resulted in the appearance of rough forms. The rough form was found to be stable in ordinary solid and liquid media. The rough form was found to revert to the smooth type when passed from 4 to 6 times through 2 per cent sucrose or 1 per cent glucose nutrient broth.

The S type of *Erwinia amylovora* is the more virulent. The R type is avirulent for some susceptible shrubs and only slightly virulent to green pear fruits and succulent tips of pear seedlings.

R, as well as intermediate types, was obtained from old infections in nature.

Other variants were observed, including types with rhizoid and translucent colonies. The translucent type grows slowly in nutrient broth, requires frequent transplants in order to be perpetuated, and is avirulent to pear, apple, and certain shrubs.

Ten per cent sucrose broth decreases the invasive capacity of the culture but not by increasing the rough forms, while 2 per cent sucrose restores both attenuated and rough forms to the virulent state.

The influence of varying sugar concentrations, such as occur in the nectar of fruit-tree blossoms, and the dissociation of the organism, are believed to have an important bearing upon the rise and decline of epiphytotics of fire blight.

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VARIABILITY OF POLYPORUS SCHWEINITZII IN CULTURE[1]

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INTRODUCTION

Slight cultural differences within various species of wood-attacking fungi have been briefly mentioned by Long and Harsch (8) and by Fritz (3), but these workers, primarily interested in the diagnosis of decay, attached little importance to the variability they encountered. Schmitz (14) has described definite morphological and physiological differences between 4 isolations of *Fomes pinicola* (Sw.) Cke. Mounce (11), also working with *F. pinicola*, has discussed differences between cultures from a number of sources and ascribes these differences to individual variation rather than host influence. Aside from these studies, intraspecific variability in the Polyporaceae seems to have received little or no detailed attention, although the possibility of its occurrence often has been suggested.

When *Polyporus schweinitzii* Fr. was found causing a destructive root rot in 20- to 25-year-old plantings of northern white pine, *Pinus strobus* L., on unfavorable sites near Springwater, New York,[3] the possible implication of a heretofore unreported parasitic strain of either native or foreign origin could not be ignored. Cultures of this fungus, therefore, were requested from several American and foreign sources, and a number of isolations were made from diseased white pines at Springwater. A few isolations also were made from white pine and larch in New England. As this material accumulated it became increasingly apparent that there was no morphological basis for subdividing the species into local, parasitic, or host-specialized strains. Some of the cultures resembled each other rather closely, and all of them had a few characters in common, but with the exception of those taken from within a few feet of each other no two were identical, nor did those from any given host or locality manifest any special similarity.

In the past, *Polyporus schweinitzii* has been known principally as a cause of heart rot in butts of over-mature trees; with the disappearance of

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[2] This study was begun during the tenure of a Morris Arboretum Fellowship in the Department of Botany of the University of Pennsylvania, and was completed at the Morris Arboretum with the assistance of a fellowship granted by the Charles Lathrop Pack Forest Education Board. The writer also wishes to acknowledge his indebtedness to Dr. H. H. York and Dr. Conway Zirkle, to Edith Adams Childs for laboratory assistance, and to the many workers who furnished cultures.

[3] York, H. H. A study of resinosis and root rot in forest plantings of *Pinus strobus* and *P. resinosa*. Unpublished manuscript.

virgin stands it was expected to become relatively unimportant. Its destructiveness at Springwater and in a Douglas fir plantation near Biltmore, North Carolina, (6) suggests, however, that it may constitute a serious obstacle to the use of coniferous species in artificial reforestation, at least on certain sites. An investigation of its variability has, therefore, been undertaken to determine to what extent specialized strains may be responsible for damage to young trees. Certain preliminary observations are presented here. Final conclusions will be possible only when the results of field studies, already inaugurated, become available.

SOURCES AND DESCRIPTIONS OF CULTURES

It was the writer's practice to make several isolations from each sporophore or infected tree from which a culture of the fungus was desired. More than 100 isolations, representing 25 sporophores and 9 trees, were thus secured and cultured on nutrient agar under closely comparable conditions. With 2 exceptions, isolations from any given tree or sporophore were indistinguishable from one another, but differed more or less distinctly from those from any other source. It, therefore, was assumed that similar isolations from the same source were derived from the same individual mycelium, *i.e.*, were members of the same clone, and that dissimilar isolations were derived from genetically different mycelia. These mycelia, together with those obtained from other workers, will be referred to by the following numbers:

1 to 30, inclusive. From *Pinus strobus*; Springwater, N. Y.
31. From *Pinus strobus* (lesion on 0.5 cm. root of an 8-year-old planted tree); Honeoye Lake, N. Y.
32. " " " ; Weston, Ontario.
33. " " " ; Lake Timagami, Ontario.
34. " " " ; Harvard Forest, Petersham, Mass.
35. " " " ; Brattleboro, Vt.
36. " " *silvestris*; Stralsund, Germany.
37. " " " ; Skjaerningsfjell, Ringebii, Norway.
38. " " " ; Great Britain.
39. " " *rigida*; Medford, N. J.
40. " " *mughus;* Central Experimental Farm, Ottawa, Ontario.
41. " *Picea canadensis*; Lake Timagami, Ontario.
42. " " *sitchenis*; Moresby Island, British Columbia.
43. " " " ; Gahrenberg, Germany.
44 and 45. From *Pseudotsuga taxifolia*; Berlin, Germany.
46. From *Larix europea*; Island of Visingsö, Lake Vetter, Sweden.
47. " " *laricina*; Franconia, N. H.
48. " *Thuja plicata*; Vancouver, British Columbia.

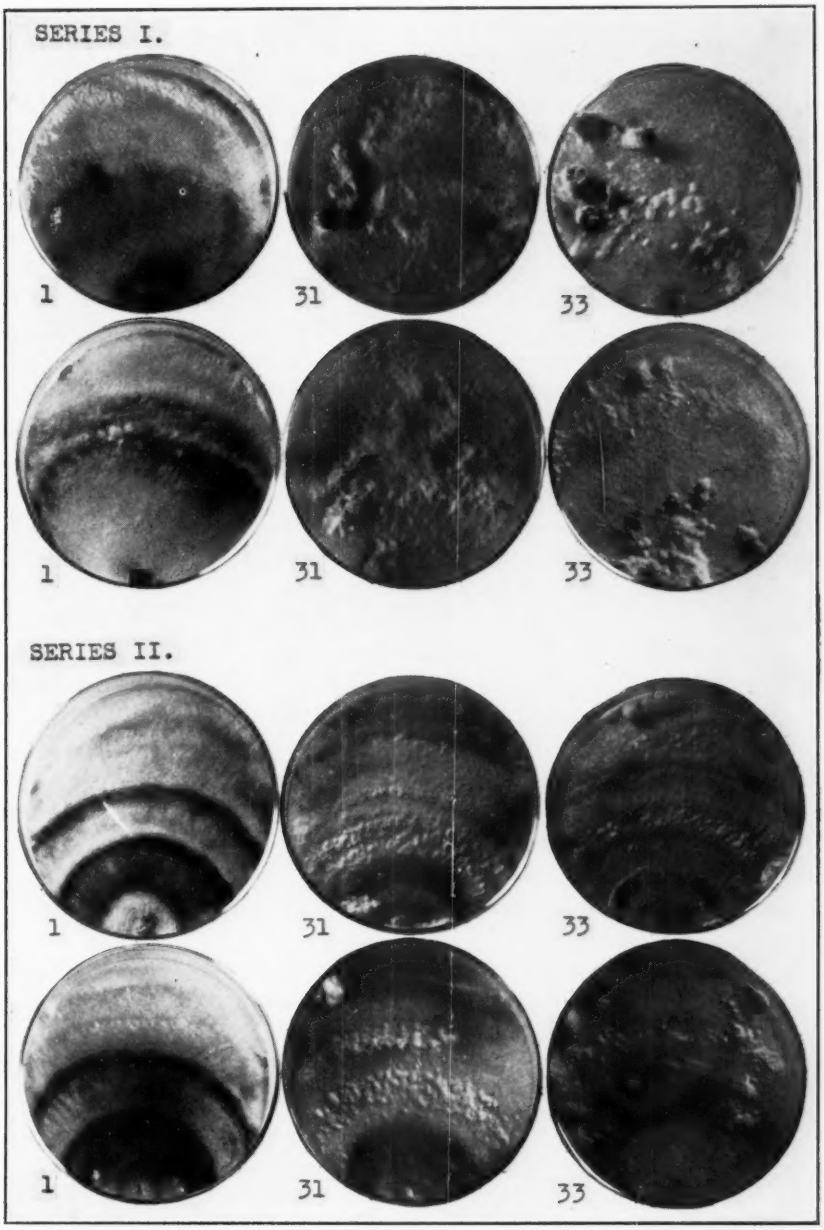

FIG. 1. Cultures showing differences between individual mycelia and between cultures of the same mycelium exposed to different environments, and similarities between parallel cultures of each mycelium. Numerals refer to the numbers given the various mycelia on pages 30 and 32.

49. Host not specified; Timagami Forest Reserve, Ontario.
50. Host not specified; Kyota, Japan.

One isolation of each mycelium was maintained as a stock culture from which all subsequent cultures of that mycelium, except when otherwise noted, were derived.

Cultures of these mycelia were under the writer's observation for periods of from 6 to 20 months, and in no instance was any alteration of cultural characters detected. At the same stage of development, cultures of any given mycelium differed in appearance only when they had been subjected to different environments. This is shown in figure 1, where growth characters of each of 3 representative mycelia are seen to be consistent in duplicate cultures raised under the same conditions (*i.e.*, in the same series) but are not consistent in cultures of the same mycelium in different series. The appearance of a given mycelium cultured in a given environment was determined entirely by the inherent nature of that particular individual mycelium and by the factors of that particular environment; *i.e.*, the cultural history of the material from which transfers were made had no appreciable effect upon the characters of the derived cultures.

Schmitz (14) and Mounce (11) have described and illustrated the line of demarcation that usually develops when different mycelia of *Fomes pinicola* approach each other on the same artificial substratum. An apparently identical reaction occurs between mycelia of *Polyporus schweinitzii*. When isolations from different sources were subcultured on the same slant or plate of nutrient agar, a well-defined dark line about 1 mm. wide usually appeared between them soon after the advancing margins had come in contact. This line did not form between subcultures taken from a single isolation or from similar isolations from any single source, nor did it form when monosporous mycelia were paired with other monosporous mycelia of either the same or different ancestry.

Where not otherwise specified, cultures were made on nutrient agar consisting of 20 g. of malt extract (either Fleischmann's Diamalt or Trommer's Diastasic) and 20 g. of agar per litre of distilled water. Sterilization was effected by autoclaving for 15 minutes at a pressure of 15 pounds. Growth was somewhat more luxuriant and fruiting was more frequent on media made with Diamalt. Trommer's extract was variable, some bottles being caramelized to a much greater extent than others, but the line of demarcation formed between mycelia from different sources was generally darker and more distinct when this brand of nutrient was used. In other respects these two brands of malt extract did not differ perceptibly in their effect on growth characters.

As Fritz (3) has remarked, *Polyporus schweinitzii* is quite sensitive to

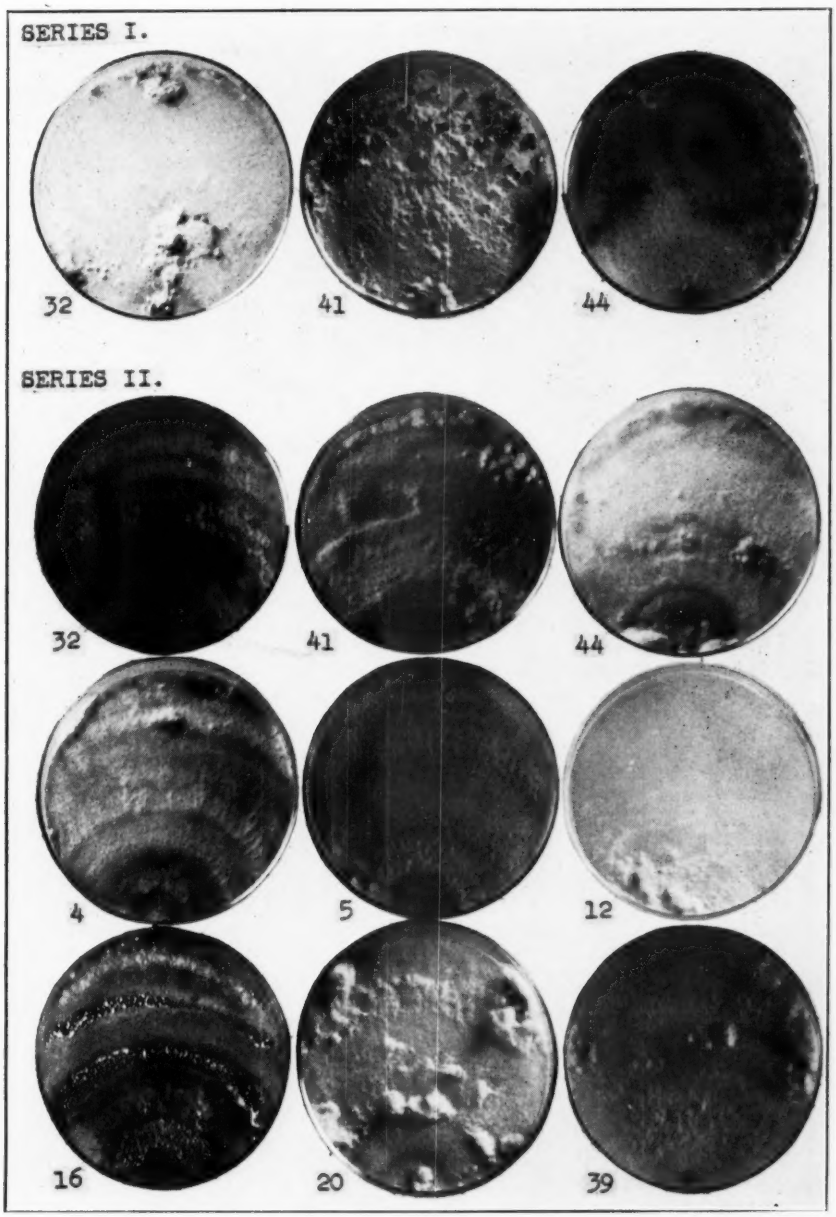

FIG. 2. Cultures showing differences between individual mycelia. Note sporophores in Nos. 16 and 41.

TABLE 1.—*Descriptions of some cultures of representative mycelia*

No.	Series	Color and character of growth			Concentric zonation
		Near inoculum	Intermediate region	Farthest from inoculum	
1.	I.	Sienna to olive brown; short pile (occasionally with 1 or 2 indistinct concentric zones of longer and darker pile).		Pale and sparse.	Indistinct. (Fig. 1).
	II.	Dark greenish-brown; uniform and moderately long pile.	Brown; uniform and moderately long pile.	Light red brown to lemon yellow; uniform and moderately long pile.	Quite distinct. (Fig. 1).
2.	I.	Yellow and light brown; fairly even mat.		Pale and sparse.	None.
	II.	Mostly greenish or reddish brown, with some lemon yellow to creamy yellow; regular and moderately long pile (occasionally slightly massed, tufted, or fluffy).			Fairly distinct.
3.	I.	Red brown; numerous very small tufts (some areas with short and fairly regular pile).		Lemon yellow.	Indistinct.
	II.	Light orange brown; fairly regular pile becoming very slightly and uniformly tufted.	Pale to lemon yellow; sparse to moderately long and irregular pile becoming slightly fluffy near edge of culture.		Fairly distinct.
4.	I.	Yellow brown (sometimes with one or two concentric zones of lighter yellow); short, matted pile.		Pale to lemon yellow.	Fairly distinct.
	II.	Olive to greenish or reddish brown; moderately long and uniform pile.			Fairly distinct. (Fig. 2).
5.	I.	Pale lemon yellow to reddish brown; an uneven mat.			Vague.
	II.	Yellow to orange; moderately long and irregular pile (some concentric zones of tufted, compacted, or fluffy growth.			Generally indistinct. (Fig. 2).
7.	II.	Mostly rather pale but with 2 or 3 fairly distinct concentric zones of lemon yellow to light brown; sparse except in regions of darker color, where regular pile is fairly well developed.			Fairly distinct.
12.	II.	Lemon yellow; short and irregular pile with some semicompact or slightly fluffy growth.	Pale and sparse.	Very pale; very sparse.	None. (Fig. 2).
18.	II.	Zones of reddish brown, short, and regular pile alternate concentrically with zones of dark yellow to orange, longer (sometimes slightly fluffy) pile.		Lemon yellow; moderately long and fairly regular pile (sometimes slightly fluffed).	Quite distinct.

TABLE 1.—(*Continued*)

No.	Series	Color and character of growth			Concentric zonation
		Near inoculum	Intermediate region	Farthest from inoculum	
31.	I.	Dark, mottled, yellow brown; irregular, matted pile with frequent fluffy growth.		Pale and sparse.	None. (Fig. 1).
	II.	Greenish to reddish brown; rather long and irregular pile.	Dark creamy yellow; small to medium large rounded tufts.	Pale to yellow green (with some reddish brown); sparse, irregular, or fluffy.	Fairly distinct. (Fig. 1).
42.	I.	Pale and sparse.	Pale and sparse.	Generally pale and sparse but with numerous long, cobwebby, lemon yellow to brown wefts or fluffy masses.	None.
	II.	Pale and sparse.	Pale and sparse.	Generally pale and sparse but with zones of loose, creamy wefts and some creamy to yellow green fluffs at edge of culture.	Rather vague.
44.	I.	Pale to yellow to reddish brown; very thin mat with short, sparse, pile (some lemon yellow, fluffy growth at edge of culture).			None. (Fig. 2).
	II.	Dark cream to orange; irregular (sometimes fluffy) pile.	Light to dark yellow green; pile irregular (fluffy in some central areas and near edge of culture).		Indistinct. (Fig. 2).
47.	I.	Yellow brown to reddish brown; short, irregular, pile and occasional small yellow fluffs.	Generally pale and sparse but usually with some pale lemon yellow growth near the edge of the culture.		Vague.
49.	II.	Greenish yellow, orange, and reddish brown; fairly long and regular pile (with a few small tufts).	A narrow zone of creamy, moderately fluffy growth.	Creamy to yellow green; sparse pile with some fairly well developed fluffy growth.	Rather indistinct.

light, growing more slowly and displaying darker and more varied coloration when exposed, even for very short periods, to indirect daylight than when kept in darkness. High humidity stimulates the development of fluffy superficial growth. Temperature, concentration and kind of nutrient, etc., also affect the appearance of cultures. The continuous nature of these environmental variables and the apparently great number of hereditary factors involved make it impracticable to describe in detail all the mycelia studied. Partial descriptions of a few representative mycelia cultured in Petri dishes are presented in table 1, however, to indicate the range and kind of cultural variations encountered during the study. In this table, and in figures 1 and 2, series I consists of cultures stored at a constant temperature of 22°[4] and exposed to electric light, but never to daylight, for a few seconds every 12 hours, while series II consists of cultures stored at room temperature (10° to 25°) and exposed to diffuse daylight for a few seconds every 4 or 5 days. Descriptions of series I were taken on the 19th day and photographs on the 40th day of growth. Descriptions and photographs of series II were taken on the 29th day of growth. From 2 to 20 cultures were made of each mycelium in each series. Cultural characters of each mycelium in each series were consistent in all cases where not otherwise indicated.

From the descriptions in table 1, and from the photographs in figures 1 and 2, it is apparent that differences between mycelia may sometimes be large enough to make difficult the diagnosis of wood decay by cultural methods. Identification may be facilitated by culturing on agar slants in ordinary test tubes and by storing cultures where they will be exposed to daylight of low intensity, since these conditions promote the formation of the more or less leathery mat and hasten the appearance of the "range of yellow tints" (3) typical of this species, but, even under these conditions, occasional mycelia are not readily identifiable on the basis of their cultural characters. The writer has not investigated the microscopic features of the various mycelia to a sufficient extent to determine their reliability as criteria of specific identity. Hasty and superficial observations indicate, however, that differences in microscopic characters are neither so great nor so frequent as are differences in gross cultural characters.

A number of mycelia were noticeably destructive to the agar, in some spots lowering the level of the surface 3 mm. or more below that of the rest of the culture (Fig. 3, 40K). Such lowering usually was confined to the region near the inoculum, but sometimes occurred in other portions of the culture. This destructive effect upon the agar, and the particular region in which destruction was visible, were quite as characteristic of certain mycelia as were the color and character of growth in culture. In cultures

[4] All temperatures recorded in this paper are in °C.

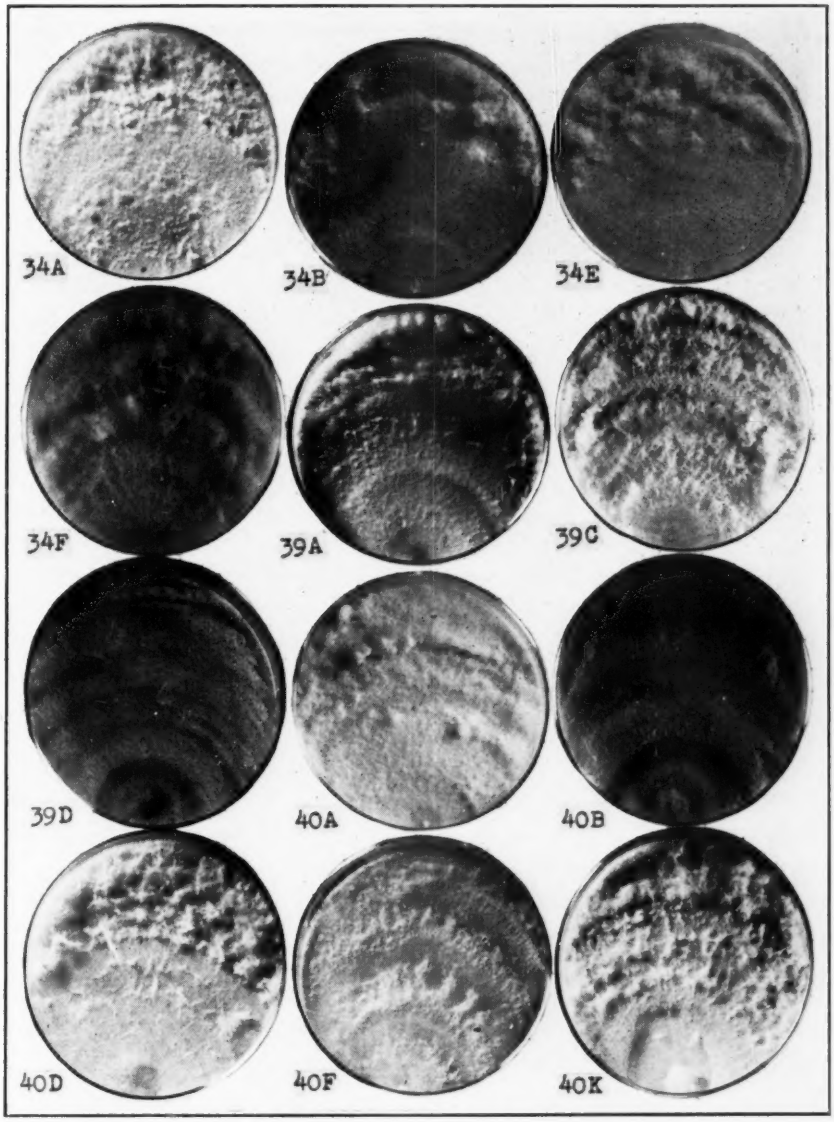

FIG. 3. Cultures showing individual differences between monosporous mycelia. Note sporophores in No. 40B and destruction of agar near inoculum in No. 40K.

of mycelia that did not alter the level of the surface of the substratum the agar retained its original gelatinous consistency until dry, but mycelia that caused any perceptible lowering of the agar surface also rendered the rest of the agar definitely crumbly, even in regions not adjacent to spots of visible destruction. Schmitz (14) has shown that some mycelia of *Fomes pinicola* secrete larger quantities of certain enzymes than are produced by other mycelia of the same species. The phenomenon just described suggests that mycelia of *Polyporus schweinitzii* also differ in enzyme production.

In only one instance during the entire study did repeated isolations from the same source fail to yield identical cultures. Two isolations each of Nos. 23 and 24 were made from a single sporophore. These mycelia differed so markedly in appearance as to be distinguishable from each other during very early stages of development. No. 23 was in no way noteworthy, displaying the usual cultural characters of the species and differing from most of the other mycelia only in relatively minor details. No. 24, however, became established very rapidly at ordinary temperatures and quickly filled the space above the agar slants with a permanently fluffy mass of hyphae. Portions of this mass ranged from a faint lemon yellow to a very light red brown, but most of it remained white throughout the life of the culture. In many respects this individual resembled some of the single-spore cultures described in a subsequent section.

No. 47, the only mycelium found in more than one individual host, was isolated several times from each of 3 wind-thrown larches separated from each other by distances of 20 to 30 feet. Since previous experience had shown that even neighboring hosts of the same species usually were infected by different mycelia, isolations from each of these trees were kept under close observation for about 6 months. At no time during this period were any greater differences detected between them than were to be found between parallel cultures derived from any single mycelium, nor was a line of demarcation formed when they grew together on a common substratum. Fruiting bodies have not been produced by these isolations and the possibility that they represent different mycelia belonging to a definite and perhaps specialized strain, therefore, has not been tested. Their homogeneity suggests, however, that a single mycelium has spread vegetatively to trees near the one originally infected.

RATE OF GROWTH ON NUTRIENT AGAR

During the growth of the cultures in series I, described in the preceding section, the radius of each culture was determined every 12 hours by measuring along the diameter of the Petri dish from the edge of the inoculum to the margin of the colony. Table 2 contains growth data derived from these measurements for 15 representative mycelia. (Nos. 4a and 35a were started

TABLE 2.—*Growth of representative mycelia in series I*

Mycelium No.	Days since establishment of cultures								Diff. between max. and min. on 8th day
	1	2	3	4	5	6	7	8	
	Average radius of mycelium—mm.								*mm.*
1.	11	17	23	28	35	43	51	58	7
2.	10	16	21	27	33	39	47	55	2
3.	10	16	22	29	37	45	53	61	8
4.	10	16	21	28	34	41	48	53	13
4a.	11	17	23	29	34	40	46	54	9
5.	10	16	23	30	37	44	52	60	3
31.	10	16	22	28	36	44	52	60	3
35.	10	15	21	27	33	40	47	55	3
35a.	10	15	21	27	34	40	48	56	3
38.	9	18	28	37	46	55	64	74	3
40.	8	11	14	18	22	25	30	34	13
41.	11	18	25	33	42	51	60	69	4
42.	12	21	30	38	46	55	64	74	6
43.	12	19	27	33	40	50	59	68	2
46.	13	22	30	38	46	54	62	70	2
48.	10	15	21	28	34	42	49	56	4
50.	10	16	21	27	34	41	49	56	6

one week later than the rest of the series, but were otherwise identical in origin and treatment with Nos. 4 and 35, respectively). Averages for each mycelium are based on 4 cultures of that mycelium. The principal difficulty encountered in studies of growth rates in this species was the difference in time required by the various mycelia for establishment on new substrata. In most instances, this difference was correlated with the character of the growth in cultures from which transfers were made. Other factors being equal, establishment was rapid when the transferred material included fluffs of loose aerial hyphae; less so when the aerial growth was compacted or matted; and slowest when the projecting hyphae were sparse and short. Mycelia that were to be transferred for comparative purposes were invariably cultured under the same conditions, but, because of inherent morphological differences, it was impossible to secure the same type of material for transfer from different mycelia. For this reason, the age of each mycelium in table 2 is computed from the time when the 4 cultures of that mycelium attained an average radius of 5 mm. (arbitrarily taken to indicate establishment) instead of from the time the transfers were made. Averages have been calculated, where necessary, by straight-line interpolation between the original semi-daily averages. The close correspondence between Nos. 4 and 4a, and between Nos. 35 and 35a, shows that, within rather wide limits, the

age of the agar is not an important factor, and that the arrangement of the data in this table may therefore be considered legitimate.

Differences between mycelia are clearly apparent in table 2. It is evident that Nos. 38, 41, 42, 43, and 46 have attained a greater radius and are growing more rapidly than the other mycelia, and that the growth rate of No. 40 is relatively low. Relative growth rates of mycelia cultured at higher or lower temperatures than series I were, however, frequently not consistent with the classification suggested by this table. For example, at 27° No. 43 grew more rapidly than No. 42, while at room temperature (Series II) No. 42 grew more rapidly than did No. 43. The cultures of No. 48 included in series II increased their average radius from 8 to 84 mm. in 14 days, while the cultures of No. 50 in the same series increased from 7 to only 65 mm. during the same period: at 27° the growth of No. 50 was appreciably more rapid than was that of No. 48. In every case where 2 mycelia differed significantly in growth rate at the extremes of the temperature range (app. 10° to 27°) to which they were exposed, but not at one or more of the intermediate temperatures, the mycelium that grew relatively rapidly at one extreme grew relatively slowly at the other. These observations indicate a difference between mycelia either in the form of the temperature-growth curve, or in the location of the maximum ordinate of this curve (*i.e.*, the optimum), or both.

Inclusion of the 3 mycelia from spruce in the group of rapidly growing individuals in table 2 is probably the result of coincidence rather than of any host-correlated relationship, since these 3 mycelia differed rather widely from one another in growth rate at other temperatures. Mycelia from Springwater, with one or two exceptions, maintained their relative rates of growth more consistently at the different temperatures to which they were subjected, and resembled each other more closely in absolute growth rates than did mycelia from other sources. This tendency toward similarity, even if not fortuitous, does not necessarily imply the existence of a specialized strain at Springwater. It may indicate only the closer degree of relationship that might logically be expected between isolations from a limited area as compared with isolations from widely separated points.

EFFECT OF ACIDITY ON GROWTH

Nutrient solutions adjusted to 9 different acidities were prepared by mixing 3 parts of 3 per cent Trommer's malt solution with 2 parts of KH phthalate buffers compounded according to the formulas of Clark and Lubs (2). The buffered solutions were placed in 38 × 200-mm. test tubes (app. 65 cc. of solution per tube) and autoclaved 15 min. at 15 lbs. pressure. The acidity of a sample from each lot was then determined potentiometrically, and the remaining tubes were inoculated with various representative my-

celia. One culture at each pH was made of each mycelium, except No. 1, which was cultured in duplicate. Twenty-three days after inoculation the depth of the mycelium and the acidity of the medium in each tube were determined (Table 3). Since in no case did the change in pH during the

TABLE 3.—*Effect of acidity on growth of mycelium of Polyporus schweinitzii*

Initial pH of buffered nutrient	Mycelium No.									Final pH
	1	1	8	31	40	42	47	49	50	
	Depth of mycelium—mm.									
2.5	9	10	5	5	8	8	6	6	15	2.6
3.0	18	18	13	12	15	15	10	10	28	3.0 to 3.2
3.5	26	27	28	30	22	34	16	25	45	3.6 to 3.7
4.0	40	43	41	44	45	46	37	39	52	4.2
4.3	42	44	50	45	46	52	50	42	52	4.5
4.7	44	46	55	39	40	50	43	42	55	4.8 to 4.9
5.1	43	43	53	36	7	48	39	46	55	5.2
5.4	22	21	28	17	9	20	20	34	33	5.4 to 5.6
5.7	10	10	10	7	4	11	7	12	15	5.6 to 5.9

growth of the fungus exceed 0.2, and since the 2 cultures of No. 1 at each acidity were reasonably consistent, the data in this table are considered approximately indicative of the effect of acidity on the growth of these mycelia. It will be seen that in every instance the optimum pH for growth was greater than 4.0 and less than 5.4, and in most cases probably lay between 4.2 and 5.0. Within these limits, however, the acidity permitting maximum growth was somewhat different for different mycelia. For example, Nos. 31 and 40 obviously made their best growth in more acid media than did Nos. 49 and 50.

RATE OF DECAY OF WOOD BLOCKS IN VITRO

Thirty-two white pine blocks (all from the same tree) approximately $\frac{7}{8}'' \times \frac{7}{8}'' \times 4''$ were air-dried in the laboratory for several weeks, given individual numbers, weighed to the nearest half-gram, placed in 38×200-mm. test tubes half full of water, and autoclaved 30 min. at a pressure of 15 lbs. They were then transferred to similar tubes, each of which contained a pure culture of the fungus growing actively in about 25 cc. of 2 per cent malt-extract solution, and stored in darkness at 22°. Two months later an additional 30 cc. of sterile distilled water was added to each tube. After a total incubation period of nearly 9 months the blocks were removed from the tubes, air-dried for 3 weeks, and reweighed. Average losses in weight, expressed as percentages of the original air-dry weights, were as follows:

Heartwood	
9 blocks inoculated with No. 1	$4.9 \pm 0.7\%$
7 " " " " 39	$3.7 \pm 0.7\%$
Difference	$1.2 \pm 1.0\%$
Sapwood	
9 blocks inoculated with No. 1	$17.7 \pm 1.0\%$
7 " " " " 39	$24.9 \pm 2.7\%$
Difference	$7.2 \pm 2.4\%$

The difference between the 2 sets of heartwood samples cannot be considered significant, since it is only slightly greater than its probable error.[5] The difference between the 2 sets of sapwood samples, however, is 3 times its probable error, and in spite of the small basis it may, therefore, be concluded that there probably is a real difference in the ability of these 2 mycelia to attack sapwood under the conditions of this experiment. Schmitz (14) presents evidence of similar differences between mycelia of *Fomes pinicola*. In his studies, as in the present instance, the relative order of destructiveness in heartwood is not the same as that in sapwood.

SPOROPHORES IN ARTIFICIAL CULTURE

Eleven mycelia, including isolations from both sporophore tissue and decayed wood, fruited *in vitro* during the course of these studies. Nine of the 11 fruited under conditions to which most or all of the 39 sterile mycelia also were exposed, while the remaining 2 produced sporophores only under conditions to which less than half of the other mycelia were subjected. A few of the fertile mycelia fruited commonly: in others, fruiting was rare or sporadic.

In the series of wood-block cultures described in the preceding section, No. 39 formed sporophores consistently on sapwood but not on heartwood. It also fruited several times on white-pine sawdust and at the surface of cultures growing in a 2 per cent solution of malt extract. The only other mycelium cultured on these media was No. 1, which failed to fruit on any of them.

On Trommer's malt agar, Nos. 39 and 50 produced one sporophore each. Nos. 23, 30, 34, and 41 occasionally formed a few sporophores, and No. 40 fruited rather commonly. On this medium, sporophores usually appeared only in rather old cultures. Fruiting, therefore, might have been less sporadic had it been possible to retain the cultures longer.

Diamalt agar was apparently a somewhat better medium for sporophore

[5] Standard deviation $= \sqrt{\frac{\Sigma(fd^2)}{n-1}}$

Probable error of average $= \frac{0.6745 \text{ S.D.}}{\sqrt{n}}$

Probable error of difference $= \sqrt{P.E._a{}^2 + P.E._b{}^2}$

production. No. 1 fruited rarely; Nos. 9, 13, 23, and 41 fruited occasionally; and No. 16 fruited fairly regularly on this medium. No. 34 never produced sporophores in test-tube cultures but did so invariably in Petri dishes except when the layer of agar was quite thin. The most consistent and prolific fruiting encountered during the entire study was afforded by No. 40. This mycelium commonly produced sporophores in test-tube cultures: in Petri dishes, sporophore rudiments appeared soon after the surface of the agar was overgrown and functioning sporophores were often present in cultures only 3 weeks old.

The largest sporophores were those of Nos. 39 and 50, which averaged 1 cm. in diameter and ranged from 5 to 15 mm. in thickness: the smallest were those of No. 40, which averaged 3 mm. in diameter and 2 mm. in thickness. Color varied from yellow (Nos. 34 and 40) to reddish brown (No. 50) or dark brown (No. 39). Neither stipes nor recognizable pilei were present in any instance. Sporophores that developed above the culture medium consisted principally of short spines (produced by the majority of the fertile mycelia), small and disarticulated lamellae (Nos. 34 and 40), or longer hydnoid processes, frequently once- or twice-dichotomized (formed only by No. 39). Pores occurred infrequently, and then only in sporophores borne laterally, as on wood blocks and vertical agar slants, or vertically, as in inverted Petri dishes. Average pore dimater varied from less than 0.5 mm. in No. 40 to slightly more than 1 mm. in No. 39. Small pores were approximately circular but the larger ones were quite angular. Young fruiting bodies that had recently become spiny or lamellate could sometimes be induced to form pores by reversing their orientation with respect to gravity. Neither sporophore production nor pore formation appeared to depend on exposure to daylight. The microscopic structure of hymenia in artificial culture did not differ essentially from that of hymenia developed under natural conditions. Sporophores formed by different mycelia displayed some microscopic dissimilarities—for example, cystidia of No. 34 were relatively large and sometimes terminally swollen, while those of No. 40 were smaller and of more nearly uniform diameter throughout. These variations, however, were no greater than those occurring normally in "wild" sporophores. It seems probable that the well-known variability of the sporophores of many species of Polyporaceae under natural conditions (9, 15) is simply another example of differences between individuals.

MONOSPOROUS AND POLYSPOROUS MYCELIA

Basidiospores of *Polyporus schweinitzii* are so small and fragile that it is extremely difficult to secure uninjured single spores by Hanna's dry needle method (4), even with micromanipulation apparatus. Progressive dilution of material from spore prints is much less laborious, but the serviceability

of this method is impaired by the persistence with which many of the spores remain aggregated in small groups throughout the process of dilution. The procedure devised by Mounce (11),[6] however, proved quite satisfactory and in a slightly modified form was followed in obtaining all single-spore cultures except those from No. 39, which were secured by dilution. Polysporous cultures were obtained by scraping an uncontaminated spore print with a sterile spear-pointed needle and planting the material thus obtained on a slant of nutrient agar. Both monosporous and polysporous cultures were derived in all cases from spores produced by pure cultures of the fungus.

Fresh spores germinated readily, and numerous monosporous cultures were obtained from Nos. 34, 39, 40, and 50. These monosporous cultures resembled the stock mycelia in many respects. The mat developed on the surface of the agar seemed, however, generally thinner and more delicate, and superficial growth was often much fluffier. The following, cultured under the same conditions as series II, are fairly representative (Figure 3):

No. 34A.[7] Orange brown and rather irregular pile with numerous short, fluffy, pale lemon yellow tufts (Fig. 3).

No. 34B. Growth sparse and pale near inoculum; becoming pale yellow and loosely fluffed at other side of culture (Fig. 3).

No. 34D. Orange to dark olive brown pile near inoculum; pale lemon and loosely tangled fluff at other side of culture.

No. 39A. Reddish brown near inoculum; reddish brown pile with numerous short tufts and fluffy cream-color masses at other side of culture (Fig. 3).

No. 39B. Reddish brown near inoculum; a mixture of light orange and dark cream at far side of culture.

No. 39C. Zones of pale, sparse growth alternate with zones of orange pile near inoculum and with zones of cream-color fluffy growth at far side of culture (Fig. 3).

No. 40A. Growth near inoculum mostly pale and scantily fluffy; rest of culture consists of light to dark yellow fluff (Fig. 3).

No. 40B. Sparse and pale near inoculum, with some destruction of agar; rest of culture dark lemon yellow to olive or red brown (Fig. 3).

[6] "A sporophore was removed from a culture with sterile forceps and quickly fastened to the cover of a sterile Petri dish by means of a drop of melted agar. Then the cover was removed from a Petri dish containing lactose gelatine, the cover bearing the sporophore was substituted, and slowly rotated. Then the cover was replaced and as many Petri dishes as desired were inoculated in this way. The plates were kept at room temperature for from four to five days. By that time, the germinating spores appeared as tiny depressions in the surface of the clear gelatine. A circle was drawn around each mycelium, it was examined under the microscope, and if it proved to be monosporous, and if there were no other spores within the circle, it, surrounded by a small square of gelatine, was removed with a sterile spear-shaped needle, placed on a potato-dextrose or malt agar slant, and grown at room temperature."

[7] Numeral refers to parent mycelium—*e.g.*, No. 34A is the first monosporous mycelium secured from No. 34.

No. 40C. Mostly sparse and pale near inoculum; rest of culture raggedly fluffy and pale yellow to dark creamy yellow or light orange.

No. 40F. Zones of sparse and pale growth alternate with zones of short, lemon yellow tufts (Fig. 3).

No. 40K. Most of agar destroyed in immediate vicinity of inoculum; rest of culture consists of light to dark yellow fluff (Fig. 3).

From these descriptions, and from table 4 and figure 3, it will be seen

TABLE 4.—*Growth of representative mycelia of both monosporous and natural origin (Series II)*

Mycelium No.[a]	Age of cultures—days				Diff. between max. and min. on 16th day
	7	11	16	21	
	Average radius—mm.				*mm.*
1.	15	27	49	77	2
3.	2	13	41	69	4
4.	18	33	64	82	4
34.	6	17	41	63	3
34A.	22	39	65	[b]	0
34C.	18	36	67	[b]	8
34D.	22	40	68	[b]	3
34F.	16	28	48	63	4
39.	8	25	56	80	0
39A.	9	27	55	79	2
39C.	12	26	54	74	2
39D.	10	23	38	54	8
40A.	12	32	63	83	2
40B.	13	31	60	81	7
40E.	12	32	69	[b]	2
40F.	13	30	56	76	7
40K.	11	29	62	[b]	1
41.	13	32	63	82	7
43.	6	19	39	62	9
46.	7	23	56	90	2
48.	8	24	51	84	6

[a] Data for each mycelium are based on 2 cultures.

[b] Entire surface of culture overgrown (radius greater than 90 mm.).

that differences between monosporous mycelia are of the same general type and of about the same order of magnitude as those between isolations secured from different sources in the field. Cultural differences are ill-adapted to quantitative expression, and an exact comparison of the extent of variability in the different groups of monosporous mycelia of common descent is, therefore, impracticable; in general, however, it may be said that mycelia having the same immediate ancestry appeared to differ less than did those of more diverse descent. Individual differences were apparent, however, even be-

tween mycelia descended from the same sporophore. When cultured under the same conditions, hyphal-tip transfers from opposite sides of a given monosporous colony were culturally identical.

Twenty-four of the 41 monosporous mycelia secured from No. 40 produced sporophores. From one of these fertile monosporous mycelia (No. 40L) 33 single-spore cultures were made, of which 30 produced sporophores. These third-generation cultures resembled each other very closely, much more so than did those of the preceding generation, but could not be considered identical in appearance. The only other sporophores that appeared in either monosporous or polysporous cultures were developed by a few descendants of Nos. 39 and 50. Some of these were presumptively of single-spore origin, but a few of the supposedly monosporous cultures (including the fertile ones) derived from No. 39 showed striking similarity to polysporous cultures from the same mycelium: In view of the defects of the dilution method (used with spores from this mycelium only) the monosporous origin of these cultures is doubtful.

Polysporous cultures from Nos. 34 and 40 were similar in general appearance to isolations secured in the field, but polysporous cultures from Nos. 39 and 50 displayed none of the gross characteristics of *Polyporus schweinitzii*. The hyphae in the latter two were hyaline, giving cultures on malt agar a dirty grey appearance, and were almost entirely confined to the medium within 1 mm. of the surface of the agar slants. Polysporous cultures from No. 39 produced a few short aerial hyphae that looked like very fine cotton lint sparsely sprinkled on the surface of the agar. Those from No. 50 produced no aerial growth. In other respects these cultures were indistinguishable from one another. After repeated transfers, several of the polysporous cultures from No. 39 developed thin, irregular, dark reddish brown mats on the surface of the agar slants: such cultures invariably produced sporophores.

DISCUSSION

Morphological or cultural characters and more purely physiological qualities are not necessarily correlated (1). Nevertheless, the wide range of variability apparent between the cultures from Springwater, a range almost as great as that found between cultures from widely separated points and unlike hosts throughout the Northern Hemisphere, indicates that damage in this locality cannot now be attributed definitely to infection by a race or strain of special virulence. The planting stock used in the infected areas at Springwater came from nurseries in which seedlings from European nurseries had been transplanted, and other plantings in the immediate neighborhood have been made with stock of European origin, but the large number of different mycelia isolated from the infected trees precludes the possibility of infection having originated entirely by vegetative spread of the organism

from one or a few foci in nursery beds. Fruiting bodies of *Polyporus schweinitzii* have been rare in these plantings until quite recently.[8] It seems unlikely, therefore, that a few original infections by a virulent strain have been spore-propagated, with consequent morphological diversification of the organism, to a sufficient extent to account for the large and varied population of this species now present in these areas. Wean[9] has shown that infection may occur in very young trees, but his studies also indicate rather definitely that parasitic activity of this fungus is favored by certain conditions, such as those at Springwater, which are suboptimum for white pine. Pending the completion of inoculation studies in the field it must accordingly be concluded that the *Polyporus schweinitzii* epiphytotic in this locality probably is attributable to edaphic factors that have permitted extensive infection by native representatives of the species.

The possibilities of future specialization created by the wide variability exhibited by this fungus must not be overlooked. It may be assumed that the majority of the individuals constituting this species are now adapted to a quasi-parasitic life in the basal heartwood of older trees, but extensive infection of younger trees growing under more or less unnatural conditions might conceivably result in the gradual selection of lines best adapted to such existence and a consequent increase in the average virulence of the species to planted trees even on good sites. The likelihood of such a change and the time necessary for it to occur must remain problematical, but the immediate values involved are great enough to justify the development and adoption of preventive and control measures.

Variability of the type described in this paper is by no means peculiar to the Polyporaceae. Recent pathological literature contains numerous references to similar instances of intraspecific variability in widely separated species of fungi. The work of Magie (10) on *Coccomyces hiemalis,* Johnson and Valleau (7) on *Thielaviopsis basicola,* Palmiter (12) on *Venturia inaequalis,* and Hansen and Smith (5) on *Botrytis cinerea* may be cited as random examples. All of these workers observed differences in cultural appearance between isolations, and in some cases also found differences in such characters as growth rate, spore production, size and shape of conidia, pathogenicity, reaction to toxic substances, and reaction to acidity of the medium. None of these differences were correlated with the host or locality from which the isolations were made. Palmiter's (12) statement seems generally applicable to all of the above-mentioned species: "No two of the 36 isolations studied appeared exactly alike when grown under standardized conditions, yet duplicate cultures could easily be recognized. . . . *V. inaequalis*

[8] York, H. H. A study of resinosis and root rot in forest plantings of *Pinus strobus* and *P. resinosa.* Unpublished manuscript.

[9] Wean, R. E. The parasitism of *Polyporus schweinitzii* on seedling *Pinus strobus.* Unpublished manuscript.

is not a homogeneous species or one composed of a few well-defined forms with definite cultural and pathogenic reactions but is one made up of many strains that differ in various degrees in their morphologic and physiologic characters.'' Research on such highly heterozygous species can be of maximum usefulness only when the sampling errors inseparable from studies of varied populations have been reduced by the use of a large and representative basis. Small bases may be sufficient in species characterized by slight variability, but in most instances a high degree of variability must be assumed until investigations of numerous isolates have shown the species to be essentially uniform for the character in question. It seems probable that discrepancies between quantitative data secured by competent workers often may be charged directly to the basing of studies on only one or a few individuals from species in which wide variation may exist. For example, Lagerberg and Melin,[10] Robak (13), and Weis and Nielson (16) disagree rather widely as to the optimum pH for growth of *Fomes annosus* (Fr.)Cke. in artificial culture. This disagreement may be the result of differences in experimental conditions, but the observations of Palmiter (12) and the present writer suggest that it is caused by differences between the individual mycelia used in the various studies.

The term ''strain'' often is employed by pathologists to designate a single original isolation and its subcultures (with the exception of saltants, etc.). Its use in this particular connection is unfortunate, since it frequently leads the reader to an erroneous conception of the relationships involved, even when it does not indicate a misunderstanding on the part of the user. ''Strain'' suggests a racial grouping[11] of genotypically distinct individuals that are homozygous for one or more physiologic or cultural characters serving to distinguish them from other members of the species. In many instances the term is, of course, correctly used, but it is not applicable to any one of the different mycelia of *Polyporus schweinitzii* herein described. Genetic irregularities and the frequent occurrence of asexual reproduction obscure the individualization of fungi, but in this group, as in any other group of organisms, individuals are the concrete units into which such abstract conceptions as strains and species are ultimately resolvable. The situation is made more complex but is not fundamentally altered by such anomalous phenomena as mixochimaeras (heterocaryotic cells), ''discontinuous variations'', and physical discontinuity of individual genotypes. For practical purposes, separated fragments of an organism or new colonies

[10] Not seen by the present writer; *fide* Weis and Nielson (16).

[11] ''When the isolates are compared on various culture media and under different environmental conditions they fall into a number of groups each of which contains isolates which are alike in their cultural characters and cultural behaviour. Each of these groups constitutes a Strain or Race, and equates with Lotsy's 'Jordanon'. . . . Cultural races may be equated with biological races in the more strict sense.'' Brierley (1).

that have originated from accessory spores of the same genotype can be accorded no greater claim to separate individual ranking in Nature than *in vitro*.[12] Brierley's isolates, then, do not necessarily represent different individuals in all cases, although for the sake of convenience they must often be considered to do so. "Mycelium" has been used throughout the present paper in a sense that the writer considers equivalent to "clone". "Genotype" or, less accurately, "individual", might be similarly used. Choice of terms must frequently depend, as in the present instance, on convenience of expression and on the extent to which relationships within the species in question are known. Whatever the terminology, the essential fact remains that some species of fungi, of which *Fomes pinicola* (11) and *Polyporus schweinitzii* may be cited as examples among the Polyporaceae, are made up of individuals that differ from one another in many respects, and that investigations of such species must, therefore, include a sufficient number of individuals to permit an approximate determination of the specific average and range of variability.

SUMMARY

Cultural studies of 50 different mycelia of *Polyporus schweinitzii*, secured from various coniferous hosts and widely separated points throughout the Northern Hemisphere, show that this species is made up of individuals that differ rather widely from one another in appearance in culture, production of sporophores, growth rate on nutrient agar, reaction to acidity, and apparently also in ability to cause decay of wood. Individual differences also were found between monosporous mycelia secured from spores produced by pure cultures of the fungus. There was no evidence of the occurrence of local or host-specialized strains within this species, and it therefore seems probable that the serious damage which the fungus is causing to planted white pine near Springwater, N. Y., is attributable to site conditions rather than to any special virulence on the part of the fungus in these plantings. The differences shown to exist between individuals of this species are comparable to those demonstrated by Mounce (11) for *Fomes pinicola*, and indicate that research on such variable species can produce generally applicable results only when a large number of individuals are studied.

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STUDIES CONCERNING THE REACTION OF BARLEY TO TWO UNDESCRIBED PHYSIOLOGIC RACES OF BARLEY MILDEW, ERYSIPHE GRAMINIS HORDEI MARCHAL[1]

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INTRODUCTION

Salmon (26, 27) lists 59 species in 26 genera of grasses as hosts of *Erysiphe graminis* DC. Marchal (17) was the first to show that this species contained several "*formes spécialisées*", or physiologic varieties, differentiated by their host specialization. From further investigations by both Salmon (28, 29, 30, 31) and Reed (19, 20, 21, 23), it is evident that different varieties occur on each of the cereals, oats, barley, rye, and wheat. Moreover, distinct varieties occur on each of the following genera of grasses: Agropyron, Bromus, Dactylis, and Poa. In general, each variety is closely confined to species of a single genus. Two of the varieties, *Hordei* and *Tritici,* are known to contain physiologic races. Mains and Dietz (15) first demonstrated this for the *Hordei* variety, and Mains (14) has shown this to be true for the *Tritici* variety. The present investigation is limited to 2 hitherto undescribed physiologic races of the *Hordei* variety.

Genetical studies concerning the resistance of barley to powdery mildew are few. In 1907, Biffen (1) studied a cross between a resistant and a susceptible variety. Recent genetical investigations are those of Dietz (5), Honecker (8, 9), and Briggs (2), each of whom used different physiologic races of *Erysiphe graminis hordei* as inoculum. Briggs also studied mildew resistance in relation to other factors.

MATERIALS AND METHODS OF STUDY

Seed of the barley species, varieties, and crosses was furnished by E. B. Mains. The study of crosses was started with the F_2. The mildew was obtained from infected barley growing in the greenhouses at the Botanical Gardens of the University of Michigan.

In studies of the seedling reactions, approximately 10 to 15 seeds of each of the varieties to be tested were planted in $3\frac{1}{2}$ inch pots on the greenhouse

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bench. When the first two or three leaves were showing the plants were sprayed with a fine mist of water and then were inoculated by shaking heavily mildewed plants over them. A uniform distribution of conidia was thus obtained on all leaves. The plants were then covered with wet muslin suspended on wires over the pots. When this was done in late afternoon a fine dew occurred on the leaves the next morning. At this time the plants were often reinoculated, thus insuring a more abundant infection. The wet cloth was removed at the end of about 24 hours from the time of first covering. In warm, sunny weather it covered the plants only at night because of the excessively high day temperature.

In the winter, when the temperature was more uniform in the greenhouse, 65° to 75° F. during the day and 55° to 65° at night, good infection was obtained merely by shaking heavily mildewed plants over the varieties to be inoculated without covering the plants with wet cloth. The inoculum was obtained from seedling plants of highly susceptible varieties, such as Oderbrucker C. I. 940.

Notes were taken about 10 to 20 days after inoculation, when the mildew had reached its fullest development. As the time approached for taking such notes the plants were no longer watered from above. In this way, washing of the spores from the leaves was considerably reduced and readings were facilitated.

Five types of reaction of the host varieties were recognized, similar to those distinguished by Mains and Dietz (15), Dietz (5), and Mains and Martini (16) in their studies of the powdery mildew of barley. These types are as follows:

0. Highly resistant. Macroscopically, no mildew evident; chlorotic or necrotic spots, or smaller areas appearing as white flecks may be developed by some varieties.

1. Very resistant. Slight to moderate development of mycelium evident macroscopically, but with little or no sporulation; chlorotic or necrotic spots, or brownish areas developed by some varieties.

2. Moderately resistant. Moderate to abundant development of mycelium accompanied by a slight production of conidia; chlorotic or necrotic spots formed by some varieties.

3. Moderately susceptible. Moderate to abundant development of mycelium accompanied by moderate sporulation.

4. Very susceptible. Abundant mycelium developed, accompanied by abundant sporulation.

The "X" type of reaction often encountered by workers with cereal rusts was seldom found in tests of barley reacting to pure physiologic races of the mildew. This is essentially a heterogeneous type of infection in which several, if not all, of the types of infection from 0 to 4 are found on the same

leaf. Plants encountered in crosses between susceptible and resistant varieties that gave this or a type 2–3 reaction are listed in the present paper as "intermediate" in reaction.

A mixture of several races usually is indicated by one or more of the differential varieties giving two or more types of reaction at the same time, much as one encounters in the "X" type of reaction. In such instances pure cultures of the races present may be obtained by resort to one of several methods. Mains (12) and Newton and Johnson (18) have given an excellent discussion of the separation of individual races from cultures containing several physiologic races in certain of the cereal rusts. Much of the technique that they describe is applicable also to powdery mildew of barley.

In the present study resort was made constantly to single pustules in order to obtain pure cultures of the races under consideration. Seedlings known to be susceptible to several races were placed on the greenhouse bench, where mildewed barley also was present. Such healthy seedling plants usually were placed 10 to 30 feet from the mildewed plants and exposed for a day to conidia that were in the air of the room. They were then removed to a compartment where there was no mildew. After the usual period of incubation signs of infection appeared on the leaves as white flecks. As the inoculation had been scattered, plants with a single white fleck of mildew could be easily isolated. If other leaves of the plant also were flecked, they were removed, leaving in every case a single white fleck or mildew pustule to a plant. These selected plants were placed in separate moist chambers or compartments and kept from further exposure to conidia. When a single pustule began to sporulate, transfers of the conidia were made with the moistened blade of a spatula to other healthy susceptible plants. The mildew was then multiplied.

REACTION OF BARLEY TO PHYSIOLOGIC RACES 6 AND 7 OF ERYSIPHE GRAMINIS HORDEI

The results presented in this and the following section were obtained during investigations conducted during the winter (November–March) and spring (April–May), 1933–1934, and the winter of 1934–1935 in greenhouse studies. In winter tests, a day temperature of 65° to 75° F., and a night temperature of 55° to 65° was maintained. In the spring the temperature occasionally went as high as 90° during the day.

Two physiologic races were isolated that did not agree with those previously described. These are designated as physiologic races 6 and 7.

Differential Host Varieties for the Identification of Seven Physiologic Races of Barley Mildew

Mains and Dietz (15) used the reactions of 4 barley varieties, Black Hulless C. I. 666, Goldfoil C. I. 928, Nepal C. I. 595, and Peruvian C. I. 935, in

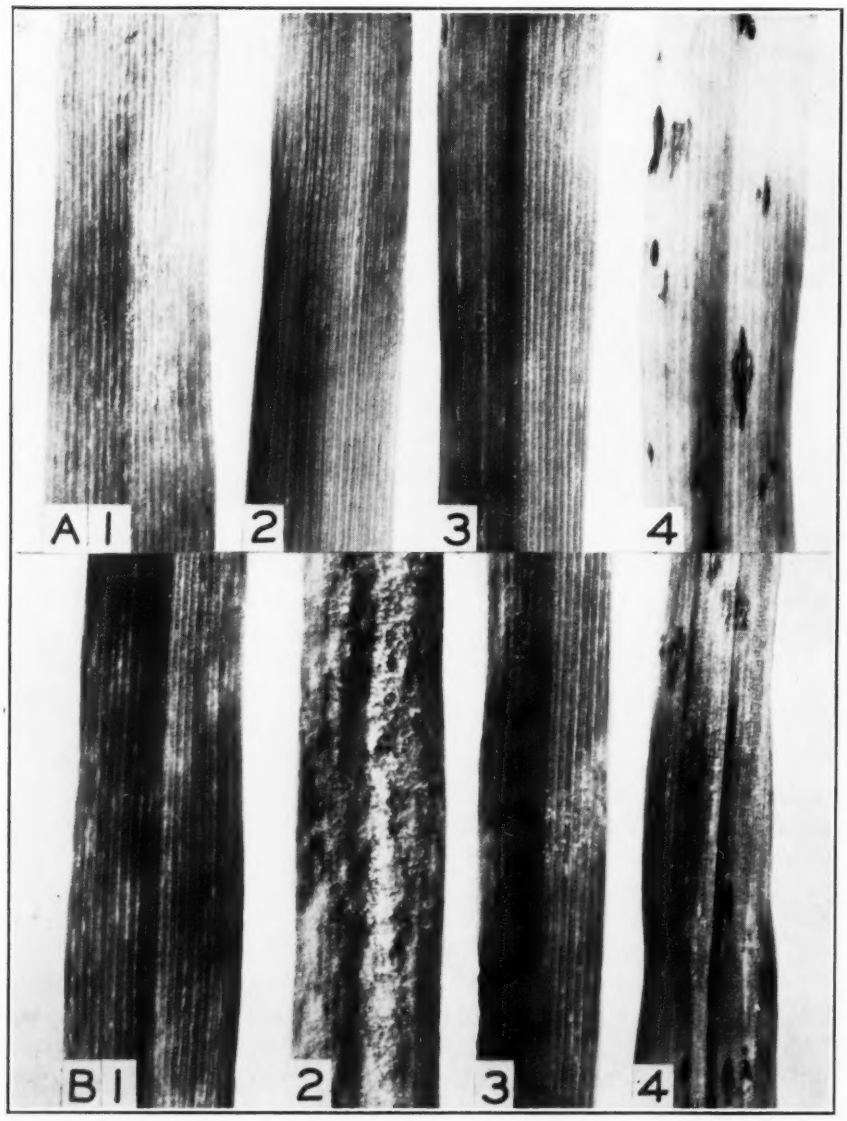

FIG. 1. Reaction of seedling leaves of four differential varieties of barley to two physiologic races of *Erysiphe graminis hordei*. ×3. A. Four varieties inoculated with p. r. 6. (1) Nepal C. I. 595, type 1, very resistant, slight development of mycelium. (2) Goldfoil C. I. 928, type 0, highly resistant. (3) Peruvian C. I. 935, type 0, highly resistant. (4) Black Hull-less C. I. 666, type 0, highly resistant with conspicuous necrotic spots. B. Same four varieties inoculated with p. r. 7. (1) Type 1–, very resistant, very slight development of mildew. (2) Type 3+, very susceptible, abundant development of mycelium and moderate sporulation. (3) Type 1, very resistant, slight development of mycelium. (4) Type 1–, very slight development of mycelium, definite necrotic spots.

order to separate the 5 physiologic races of powdery mildew they isolated. It is necessary to add Heil's Hanna 3 C. I. 682 to this list of differentials in order to separate races 6 and 1. The reactions of these 5 varieties to the 7 physiologic races are presented in table 1. Figure 1 illustrates the reactions of 4 of the differential varieties to races 6 and 7. No decided changes in reaction to either race 6 or 7 were noted between winter and spring tests, under the conditions of this study. Since no tests were conducted during the summer, in either greenhouse or field, it is not known whether these reactions will be changed under such conditions.

TABLE 1.—*Reactions of five differential varieties of barley in the seedling stage to seven physiologic races of Erysiphe graminis hordei*[a]

Variety	C. I.[b] No.	Type of reaction to physiologic race						
		1	2	3	4	5	6	7
Black Hull-less	666	0–2	1–2	3+	4	3	0–2	0–1
Goldfoil	928	0	0	0	0	4	0	3–4
Heil's Hanna 3	682	1–2	4	4	3–4	4	3–4	3–4
Nepal	595	1–2	4	4	4	4	0–1+	0–1+
Peruvian	935	0–1	1	3–4	1	4	0–1+	0–1+

[a] The data for the reactions of these varieties to races 1 to 5 inclusive were obtained from Mains and Dietz (15, p. 233).

[b] Accession number of the Division of Cereal Crops and Diseases, United States Department of Agriculture.

Reactions of Seedling Plants

Both physiologic races 6 and 7 were tested on seedlings of a number of varieties several times during the winter and spring, under the differing environmental conditions then prevalent. In general, most of the varieties reacted to race 6 much as they did to race 7 (Table 2). However, two varieties, Goldfoil C. I. 928 (Fig. 1, A2 and B2) and Hanna C. I. 906 gave reactions that differed decidedly. Both varieties were extremely resistant to race 6, giving a 0 reaction. To race 7 they were very susceptible, giving reactions of 3 or 4.

A number of varieties were extremely resistant to both races in winter and spring tests, for the most part consistently giving 0 reactions. Such varieties were Albacaete C. I. 1128, Arlington C. I. 702, Chevron C. I. 1111, Chinerme C. I. 1079, Duplex C. I. 2433, Monte Cristo C. I. 1017, C. I. 2444, M756, M757, and Psaknon B81. All, except Psaknon B81, remained green and healthy with little or no flecking, no chlorosis, and no necrosis. Psaknon showed occasional flecking and a slight browning of the leaf tissue.

A larger number of varieties were classed as very resistant, yet varying in reaction from 0 to 1. With Chilean C C. I. 1432, Chilean D C. I.

1433, Coast C. I. 276, Peruvian C. I. 1131, C. I. 1080, C. I. 2416, and Orge Fourragère B102, there was a sparse development of mycelium occasionally with a slight brown discoloration of the leaf surface under the mycelium. Sometimes this was accompanied by a slight flecking, but never with any decided necrosis of host tissue. Other varieties, such as Kwan C. I. 1016, Sulu C. I. 1022, C. I. 1021, and No. 22 B69, also gave 0 to 1 reactions, but with a conspicuous necrosis of leaf tissue. Necrotic areas several millimeters in length often were produced. In the center of some of these a little tuft of mildew mycelium occasionally was observed. Black Hull-less C. I. 666 (Fig. 1, A4 and B4) and Minsturdi M784 also were resistant, with necrosis accompanying the slight development of mycelium.

Several other varieties, classed as very resistant, varied slightly, depending on whether the tests were conducted in winter or spring. Nepal C. I. 475 and Arequipa C. I. 1256, which gave reactions as high as 2 to physiologic race 6 during the winter, gave a type-1 reaction in the spring. Juliaca C. I. 1114 varied in the winter readings, sometimes giving a type-1 reaction to race 6 and at other times as high as 3. In the spring the reactions were never higher than 1. Peru C. I. 653 sometimes gave a type-2 reaction in the winter, while in the spring this variety was slightly more resistant. The reverse, that varieties resistant in the winter were less resistant in the spring, was seldom true.

Many varieties were noted as susceptible to both races during winter and spring. In the winter some of these were moderately or highly susceptible, giving type-3 or 4 reactions, while in the spring, they were only moderately susceptible. Featherston C. I. 1120, Hooded Spring C. I. 716, and Manchuria B44 are examples. Several varieties that gave type-3 or 4 reactions in the winter gave readings as low as 2+ or 2 in the spring. Bohemia C. I. 204 was occasionally slightly more susceptible in spring than in winter. Horsford C. I. 507 and Malting C. I. 1129 are typical of the many varieties that gave type-3 or 4 reactions in both winter and spring tests to both physiologic races.

The reactions of Goldfoil C. I. 928, Hanna C. I. 906, Bohemia C. I. 204 and Palestine C. I. 939 to races 6 and 7 were interesting because these were strains of the same botanical variety of *Hordeum distichon*. Bohemia was very susceptible to both races, Palestine was very resistant to both races, but Hanna and Goldfoil were highly resistant to race 6 and very susceptible to race 7.

On seedling leaves of susceptible plants so-called "green islands" were sometimes observed, especially during the spring tests. The leaf tissue under and immediately adjoining the mildew pustules remained green, while the remainder of the leaf became yellow. In the winter, infected leaves of susceptible varieties succumbed more quickly to the mildew, and "green islands" were seldom noted.

TABLE 2.—*Reaction of barley varieties to two physiologic races of powdery mildew, Erysiphe graminis hordei, in greenhouse studies*

Variety	Accession No.[a]	Type of reaction of seedling plants to P. r. 6		seedling plants to P. r. 7		adult plants to P. r. 6	
		Winter	Spring	Winter	Spring	Winter	Spring
Abyssinia	362	1	0–1	1	1	1	0
Abyssinian	1243	1	0–1	1+	1	1	0–1
Albacaete	1128	0–1	0	0	0	1	0
Arequipa	1256	1–2	1	1+	1	1–2	0
Arlington	702	0	0	0–1	0	0–1	0
Black Hull-less	666	0–2	0–1	0–1	1–	0–1	0
Black Hull-less	1032	0–2	1–1+	1–2	1–2	0–1	
Blackhull	878	1	1–	0–1	1	0–1	0
Bohemia	204	3–3+	2–4	3+	3–4	2–3	
Bolivia	1257	1	0–1	0–1+	1	1	0
Callas	2440	2	1–2	3		0–1	0
Chevron	1111	0–1	0	0	0	0	0
Chilean C	1432	0–1	0–1	0	0–1	0	0
Chilean D	1433	0–1	0–1	0	0–1	0	0
Chinerme	1079	0	0	0	0	0	0
Coast	276	0–1	0–1	0–1	0–1	0	
Common Chile	663	1	0	0–1	0–1	0–1	
Consul	1061	1–	1–	1	1–	0–1	0
Duplex	2433	0	0	0	0	0	0
Featherston	1120	3–4	3	4	3	3–4	1+
Goldfoil	928	0	0	3–4	3+	0	0
Hanna	906	0	0	3–4	3+	0	0
Hanna	966	1	1	1	1–	1–	
Heil's Hanna 3	682	3–4	3	3–4	3+	2–3	0
Hooded Spring	716	3–4	3	3–4	3	4	0–1
Horsford	507	3–4	3	3–4	3	3	0
Horsford	610	3–4	3–4	3–4	3–	3	0
Horsford	877	4–	4–	3–4	3	3–4	2–
Juliaca	1114	1–3	1	1–3	1–2	1–2	0
Kwan	1016	0–1	0–1	0–1	0–1	0–1	0
Lion	923	1–2	1–2	1–2+	1–	1	0
Luth	972	0–1	0–1	0–1	0–1	0–1	0
Lynch	919	0–1	0	0–1+	0	0	0
Malting	1129	3–4	3	4	3	3–4	0–2+
Manchuria	2330	3–4	3–4	4	3	4	0–2
Mecknos Moroc	1379	3–3+	2+	3–4		2–3	0–1
Monte Cristo	1017	0	0	0	0	0	0
Nepal	475	1–2	1	1	1	1	0
Nepal	595	0–1+	1	0–1+	1	0–1	0
Oderbrucker	940	3–4	2–3	3–4	3	3–4	1–2
Oderbrucker	957	3	3–	4		4	1–2
Palestine	939	0–1	1–	1	1	1	0
Peru	653	1–2	0–1	1–1+	1–	0–1	
Peruvian	935	0–1+	1–	0–1+	0–1	1	0–1
Peruvian	1131	0–1	0–1	1	0–1	1	0
Purple Nepal	1373	1–	1–	1	1–	0–1	0
Quinn	1024	3	3	4		3+	1–2
Sulu	1022	0–1	0	0–1	0	0–1	0–1
	1021	0–1	0–1	0	0–1	0–1	0
Turkestan	711	0–1	0–1	1	1–	1	0

TABLE 2.—(*Continued*)

Variety	Accession No.[a]	Type of reaction of seedling plants to P. r. 6		seedling plants to P. r. 7		adult plants to P. r. 6	
		Winter	Spring	Winter	Spring	Winter	Spring
Turkestan	1080	0–1	0–1	0–1	0–1	1	0
	1347	3–3+	2–3	3	3	3	0
	2329	1	1	1	0–1		0
	2416	1	0–1	0–1	0–1	1	
	2444	0	0	0	0	0	
California Feed	B59	3	3	3–4	2	2	
Coast	B121	1	1	1+	1–	1	
Colsess	B97	1	1	1+	1–		
Lion	B33	3–4	2–4	3–4	3–	2+	
Locride	B86	0–1	0–1	0–2	1+	1–2	
Manchuria Sel. C163	B40	3–4	3–4	3–4	3	3–4	
Manchuria Sel. C164	B41	3–4	3–	3–4	3	3	
Manchuria Minn. 184	B43	3–4	3	3–4	3	3	
Manchuria	B44	3–4	3	3–4	3	3–4	
O. A. C. 21	B61	3–4	2–4	3–4	3	3–4	
Orge Fourragère	B102	0–1	1	1	1	0–1	
Orge 4th	B100	3–4	3–4	4–	3–	2	
Orge 14 J	B101	3–4	2–4	3–4	1–3	1+	
Psaknon	B81	0	0–1	0	0	0	
Sahara	B95	1	1	1–2	1–	1–1+	
Smooth Awn × Manchuria	B35	3–4	3	3–4	2+	3–4	
Smooth Awn × Manchuria	B36	3–4	3	4	2	3	
Smooth Awn × Manchuria	B37	3–4	2–3	4	3	3	
Smooth Awn × Manchuria	B38	3–4	2–3	4	3	3–4	
Virginia Hooded	B6	3–4	3–4	4	3	2–3	
No. 22	B69	0–1	0–1	0	0–1	1	
No. 305	B85	1–3	1–3	2–3	1–2	1	
Glabron	M781	3–4	3–	3	3	3–4	
Manchuria	M785	3–4	2–3	3+	3–	3–4	
Minsturdi	M784	0–1	1	1	1	1	0
Sacremento	M777	0–1	1–	0–1	1–	0–1	
Spartan	M788	3	3–	3–4	3	3	
Svansota	M786	3–4	2–3	3–4	3	3	0
	M756	0	0	0	0	0	
	M757	0	0	0	0	0	

[a] Numbers in this column *not* preceded by a letter are C. I. numbers of the Division of Cereal Crops and Diseases, United States Department of Agriculture. Numbers preceded by B are the numbers under which the varieties were received from W. L. Waterhouse (13, p. 880). Numbers preceded by M are accession numbers of E. B. Mains.

Reactions of Adult Plants

Adult plants of certain varieties also were inoculated at two seasons of the year in greenhouse studies. In these tests physiologic race 6 was used for inoculum.

The reactions of 83 varieties were obtained in the winter investigations (Table 2). Readings were made in March when the plants were in the late

shooting or heading stages. Varieties, resistant in the seedling stage, were resistant also in the adult stage. Necrosis of leaf tissue, very prevalent on seedling plants of such varieties as Kwan C. I. 1016, Sulu C. I. 1022, and Minsturdi M784, was much reduced or entirely lacking. Instead, only a browning of the host tissue was apparent. Chlorosis and flecking were more pronounced on some varieties than in seedling tests. Almost all varieties, moderately or highly susceptible as seedlings, were equally susceptible in the adult stage. On many varieties, such as Malting C. I. 1129 and several strains of Manchuria, which gave a type-3 or 4 reaction, the mildew was abundant on leaf sheaths and beards, as well as on the leaf blades. In many instances the heads were well past flowering, yet the beards were heavily mildewed. A few varieties, susceptible as seedlings, were slightly more resistant in the adult stage, giving reactions of type-2 to 3. Mecknos Moroc C. I. 1379 is an example. It was further noted that heavily mildewed plants were delayed in coming to full maturity, and the seeds in the heads were greatly reduced in number.

In another experiment, conducted with a limited number of adult plants, readings were made in November and early December, a mixture of races

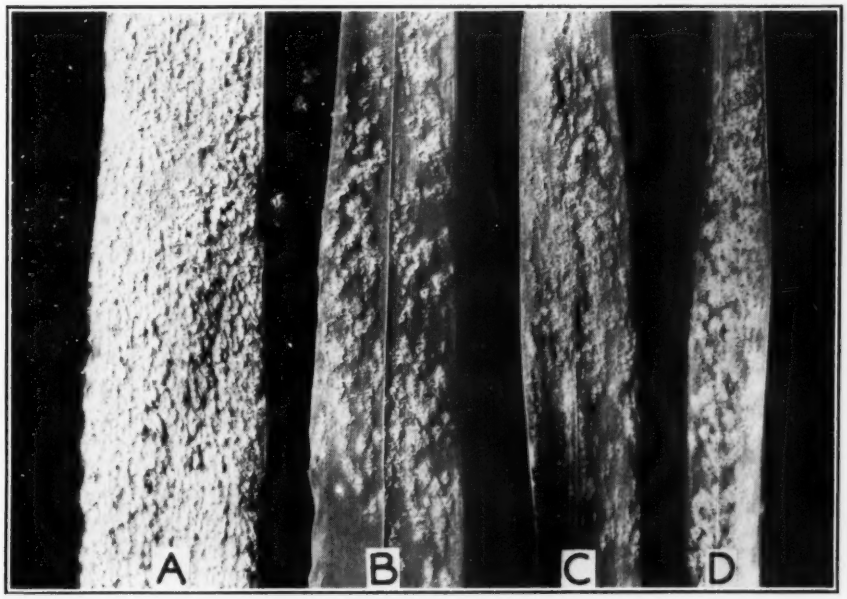

FIG. 2. Upper leaves of adult plants of four susceptible varieties of barley inoculated in winter tests in greenhouse studies with a mixture of races 6 and 7 of powdery mildew. ×1. A. Flag leaf of Manchuria M785, type 4. B. Oderbrucker C. I. 940, third leaf from top of plant, type 3. C. Virginia Hooded B6, third leaf from top of plant, type 3. D. Smooth Awn × Manchuria B36, flag leaf, type 3.

6 and 7 being used for inoculum. Results were obtained that again indicated that, given certain environmental conditions, adult barley plants are as susceptible to mildew as are seedling plants of the same varieties (Fig. 2).

In the spring series most of the varieties were in the shooting or heading stages when exposed to mildew conidia. Readings were made during the first 2 weeks of May, and the data in table 2 records the reaction noted at the time of full expression of the mildew infection. As shown in table 2, all the 48 adult varieties tested in the spring were more or less resistant. Varieties, highly resistant in the seedling stage, were equally resistant in the adult stage. Some of the varieties, showing type-3 or 4 reactions as seedlings, were highly resistant. Horsford C. I. 610 is typical. Others, such as Oderbrucker C. I. 940 and Quinn C. I. 1024, which were susceptible in the seedling stage, gave reactions ranging from 1 to 2 on the upper leaves. A slight chlorosis of leaf tissue accompanied the slight to moderate development of mycelium on many of these varieties.

Reactions of Wild Species of Hordeum

The following wild species of Hordeum were used in these experiments: Seven collections of *Hordeum murinum* L., 6 collections of *H. gussoneanum* Parl., 2 collections of *H. nodosum* L., and single collections of *H. pusillum* Nutt. and *H. jubatum* L.

The plants were first inoculated in the usual manner with physiologic race 6 in winter of 1934–1935, when they were in the 3-leaf stage. For a period of 4 weeks following this first inoculation they were constantly exposed to inoculum from heavily mildewed barley varieties that were nearby on the greenhouse bench. At no time during this period did any signs or symptoms of the disease appear on the wild species of Hordeum thus inoculated.

INHERITANCE OF RESISTANCE

Svansota M786 × Hanna C. I. 906

Both Svansota and Hanna are 2-rowed barleys. In the seedling stage in the winter, Svansota is very susceptible (type 3–4) to race 6, whereas Hanna C. I. 906 is highly resistant (type 0). The reactions of the F_2 seedlings were obtained in studies made in December, 1933. F_3 progeny tests were conducted in February, 1935.

In the F_2 test 98 plants gave a type-4 reaction, 228 a reaction of 1 or 1–2, and 118 a reaction of 0. This approximates a 1 : 2 : 1 ratio of 111 : 222 : 111, the deviations for the 3 classes being – 13, 6, 7 and Dev. ÷ P.E. 2.11, 0.84, 1.14.

F_3 progeny tests were made of 25 to 35 plants derived from each of 262 of the F_2 plants. Forty-eight susceptible F_2 plants proved to be homozygous for susceptibility. Sixty-five of the 67 individuals, classed as highly resistant (type 0) in the F_2, bred true for high resistance. The progenies

in the other two lines segregated. The misclassification of these F_2 plants is not surprising when the closeness of reaction of the two resistant classes is considered. Ten of the 147 F_2 plants, classified as type 1 or 1–2, gave F_3 progenies with only 0 reaction. The plants in the remaining 137 lines segregated.

Of 3983 F_3 plants from the 139 segregating lines 1039 gave a type-4 reaction, 2011 a reaction of 1 or 1–2, and 933 a reaction of 0. This approximates a 1 : 2 : 1 ratio of 995.75 : 1991.50 : 995.75, the deviations for the three classes being 43.25, 19.50, – 62.75 and Dev. ÷ P.E. 2.34, 0.92, 3.40.

The results obtained in the F_2 and verified by tests of F_3 lines indicate that the resistance of Hanna C. I. 906 to physiologic race 6 in the seedling stage is due to a single Mendelian factor. Apparently, the marked resistance of Hanna is not completely dominant. Heterozygous individuals, although resistant, are less so than the Hanna parent.

Featherston C. I. 1118 × Goldfoil C. I. 928

Featherston is a 6-row and Goldfoil a 2-row variety. Goldfoil is highly resistant (type 0) to race 6. The reaction of Featherston to race 6 was not obtained. The reactions of F_2 seedling plants were obtained in May, 1934, and those of F_3 lines in February, 1935. Physiologic race 6 of the mildew was used for inoculum.

In the F_2 test 37 plants gave a type-3 or 4 reaction, 70 a reaction of 2–3, and 38 a reaction of 0, 1, or 2. This closely approximates a 1 susceptible : 2 intermediate : 1 resistant ratio of 36.25 : 72.50 : 36.25, the deviations for the 3 classes being 0.75, – 2.50, 1.75 and Dev. ÷ P.E. 0.21, 0.62, 0.50.

Tests of approximately 25 to 35 F_3 plants from seed of each of the F_2 plants showed that very few of the F_2 plants were wrongly classified. One of them, classed as susceptible (3–4) in the F_2, produced offspring that segregated; while one plant, classed as intermediate (2–3) in the F_2, gave rise only to susceptible individuals. Six F_2 individuals, classed as resistant (0–2) and that had given type-2 or 2– reactions, should have been placed in the intermediate group in classifying the F_2 since the progeny in the F_3 lines from these plants segregated. These errors in classification are not surprising when the closeness of reaction of the F_2 plants is taken into consideration.

It is noteworthy that in the F_2 test, conducted in late spring in the greenhouse, heterozygous individuals gave a reaction of type 2–3, intermediate between resistance and susceptibility. In the winter, however, heterozygous F_3 plants were more resistant and gave type-1–2 reactions. Plants, homozygous for resistance and for susceptibility, showed greater stability. The individuals, homozygous for resistance, however, were not all so resistant as the resistant parent, indicating that other factors may play a part in modifying the resistance.

Of the 2466 F_3 plants from 76 segregating lines, 614 gave reactions of type 3 or 4, 1216 a reaction of 1–2, and 636 a reaction of 0 or 0–1. This approximates a 1 : 2 : 1 ratio of 616.5 : 1233 : 616.5, the deviations being – 2.5, – 17, 19.5 and Dev. ÷ P.E. 0.17, 1.01, 1.34.

The F_2 and F_3 results indicate that the resistance of Goldfoil C. I. 928 to physiologic race 6 is governed by one main factor.

The 145 F_2 plants whose seedling reactions to mildew had been ascertained were grown to maturity and classified for 2-row and 6-row arrangement. One group included 111 2-row individuals and the other contained 34 6-row plants, which approximates a 3 : 1 ratio. This is in agreement with studies of other investigators (25, 3, 4) who have found a single-factor difference between 2-row and 6-row forms, the 2-row condition being dominant.

A combination of the data concerning number of rows per head with that obtained for the inheritance of mildew reaction gives the results shown in table 3. The F_2 plants, heterozygous for resistance, are placed in the resistant class, since the heterozygous F_3 individuals from these plants tended, in the winter tests, more toward resistance than susceptibility.

TABLE 3.—*Inheritance of mildew reaction and row number in the F_2 of a cross between Featherston C. I. 1118 and Goldfoil C. I. 928*

Item	2-row		6-row		Total
	Resistant	Susceptible	Resistant	Susceptible	
Observed	84	27	24	10	145
Expected	81.56	27.19	27.19	9.06	
Deviation	2.44	–0.19	–3.19	0.94	
Probable error	4.03	3.17	3.17	1.97	
Dev. ÷ P.E.	0.61	0.06	1.01	0.48	

These data indicate a close approximation to the results that would be expected if mildew reaction and row number are inherited independently.

Arequipa C. I. 1256 × Horsford C. I. 610

The variety Arequipa is awned and Horsford is hooded. Seedling plants of Arequipa give a reaction of type 1–2 to race 6, in the winter. Under similar conditions Horsford gives a type-3–4 reaction. The data concerning the reactions of both F_2 and F_3 seedlings were obtained during the winter months in greenhouse studies.

When the F_2 plants were classified all types of reaction from 1 to 4 were encountered. One hundred and forty-two of the plants gave a reaction of type 3 or 4, and 382 a reaction of 1 or 2. This closely approximates a 1 : 3 ratio of 131 susceptible : 393 resistant, the deviation being 11, and

Dev. ÷ P.E. 1.64. The results indicate that the expression of resistance is due to a single main Mendelian factor.

In order to verify the results obtained in the F_2 generation, F_3 progenies of 25 to 35 plants derived from each of 122 F_2 plants were studied. Four of the F_2 plants were apparently erroneously classified. Twenty-five of the 122 F_2 plants were found to be homozygous for susceptibility, 34 were homozygous for resistance, and 63 were heterozygous for resistance-susceptibility. Of the 1881 F_3 plants from the 63 segregating lines, 491 gave a reaction of type 3 or 4, and 1390 a reaction of 1 or 2. This approximates a 1 : 3 ratio of 470.25 susceptible : 1410.75 resistant, the deviation being 20.75, and Dev. ÷ P.E. 1.64. The results obtained in the F_2 and F_3 indicate that one main Mendelian factor governs the inheritance of the resistance of Arequipa C. I. 1256 to race 6 of the mildew.

The 122 F_2 plants, studied for mildew reaction in the seedling stage, were matured and classified for type of lemma, hooded or awned. Some of the plants bore heads that were hooded like the Horsford parent. Some had the hoods elevated on short awns. The latter probably were heterozygous for the hooded character. Both types were recorded as hooded. Other plants were awned like the parent, Arequipa. Eighty-nine plants were hooded and 33 were awned, which is a close approximation to a 3 : 1 ratio. As shown here and as proved by other investigators (25, 3, 4), the hooded character is dominant. A single pair of factors evidently is involved.

Since the genotypic constitution of the 122 F_2 plants classified for mildew reaction was indicated by the F_3 progeny tests, the F_3 results may be used for classifying the F_2. When these data are combined with those involving the hooded and awned characters, results are obtained as given in table 4.

TABLE 4.—*Inheritance of mildew reaction and lemma character in the F_2 of a cross between Arequipa C. I. 1256 and Horsford C. I. 610*

Item	Hooded		Awned		Total
	Resistant	Susceptible	Resistant	Susceptible	
Observed	75	14	22	11	122
Expected	68.63	22.87	22.87	7.63	
Deviation	6.37	−8.87	−0.87	3.37	
Probable error	3.70	2.91	2.91	1.80	
Dev. ÷ P.E.	1.72	3.05	0.30	1.87	

Apparently mildew reaction is inherited independently of lemma character.

DISCUSSION

As early as 1902 Marchal (17) showed that the powdery mildew of grasses (*Erysiphe graminis* DC.) comprised a number of physiologic varieties, each

specialized on different grass genera. In 1930 Mains and Dietz (15) reported that the *Hordei* variety contained physiologic races distinguished by the differential reactions of varieties of barley. In the present study the occurrence of 2 additional physiologic races has been demonstrated. These have been designated as races 6 and 7. In order to separate race 6 from race 1, another barley variety, Heil's Hanna 3 C. I. 682, has been added to the list of 4 differentials used by Mains and Dietz. In 1934 Honecker (9) also reported the occurrence of 2 new physiologic races of *Erysiphe graminis hordei* in Germany. Since the reactions of 2 of the 5 differentials to the physiologic races isolated by Honecker are not known these 2 German races have not been included in table 1 of the present report, nor have they been designated by numbers.

The discovery of physiologic races of barley mildew should help to account for some of the discrepancies in the results obtained by early workers. For instance, Marchal (17, (1902)) and Salmon (28) obtained slightly different results when certain species of Hordeum were inoculated. It is probable that these investigators worked with different physiologic races. In the present study 5 wild species of Hordeum were entirely resistant to race 6. Marchal (17, (1902)) reported positive results on *Hordeum murinum* and *H. jubatum,* and Reed (20) obtained positive results on the first leaves only of *H. nodosum.* It also may be that strains of the host, differing in susceptibility, were used in the various investigations.

In the present study the reactions of varieties resistant in the seedling stage were exceedingly stable despite the fact that certain varieties were slightly more resistant in spring than in winter. Likewise, seedling plants of susceptible varieties were found to give reactions in spring very similar to those recorded in winter for these same varieties.

The effect of environmental factors on the reactions of adult barley in inoculation experiments with mildew in winter and late spring in the greenhouse is well defined. In winter, under conditions of low light intensity and lower temperatures, the varieties susceptible in the adult stage were fully as susceptible as in the seedling stage. Adult plants of varieties resistant as seedlings gave reactions very similar to those recorded in the seedling stage. In spring, however, the adult varieties, susceptible during winter and spring in the seedling stage, were moderately to highly resistant. Adult plants of varieties resistant in the seedling stage were often more resistant than seedlings of the same varieties. Instead of giving 0–1 reactions, as did many of the seedlings, the reactions were type 0. This pronounced resistance of adult plants in the greenhouse in spring may be due to increased light or higher temperatures acting on the host-parasite complex.

Graf-Marin (7) states that he never observed mildew on the leaves of mature barley plants, although seedlings of the same variety developed the

disease. He attributed the resistance of the leaves of adult barley to the fact that the haustoria of the fungus were unable to penetrate the thicker cuticle and walls of the epidermal cells of "old leaves". It should be noted that his conclusions were based on results obtained with only one variety grown in the greenhouse in the spring. It is conceivable that had Graf-Marin inoculated adult plants of this variety in winter, infection would have resulted, as it did on adult plants of susceptible varieties in the investigation here reported. In this connection it also might be noted that Miss Mackie (11) found no excessive morphological differences between leaves of resistant and susceptible barley varieties.

Several investigators (32, 33, 6, 8, 10) have observed that certain growing conditions are more favorable for barley-mildew development and the resistance or susceptibility of the host may be somewhat modified. From the results obtained in the present studies it also is evident that the reactions of some varieties may be modified by environmental factors. This is especially true of adult plants of susceptible varieties as already mentioned. With seedling plants, however, the changes were for the most part not pronounced. This conclusion is supported by the results obtained in the genetical studies.

The "green island" phenomenon, observed in spring on leaves of susceptible varieties in the seedling stage, has been noted by several investigators. Graf-Marin (7) noted "green islands" on barley leaves that had almost been killed by ultraviolet light. Reed (22) mentioned the occurrence of green areas infected with mildew. The yellowing of barley leaves beyond the infected green regions was suggestive of the secretion of toxic substances by the mildew (11). Miss Rice (24) has given a good discussion of the occurrence of this phenomenon in the rusts. In the writer's experiments the "green islands" were occasionally noted on a few leaves that died from causes other than mildew infection. Such "green islands" were very small. The best examples were observed on seedling leaves of some of the susceptible varieties in the spring tests. These leaves generally were fairly heavily infected with mildew and the "green islands" gradually became prominent as the leaf yellowed.

The genetical studies with seedling plants demonstrate that resistance is a definitely inherited character. The resistance of the 3 resistant parent plants studied, Hanna C. I. 906, Goldfoil C. I. 928, and Arequipa C. I. 1256, was shown in each case to be due to a single main Mendelian factor. The resistance of Hanna was incompletely dominant, the heterozygous individuals being somewhat less resistant than Hanna and homozygous resistant segregates. The same was true for Goldfoil. In crosses where Arequipa was the resistant parent, heterozygous individuals were not so clearly differentiated. The resistance of Arequipa is not so stable as that of Hanna or Goldfoil and is more easily varied by environmental conditions and probably is responsible

for the intergradation of the homozygous resistant and heterozygous groups. Honecker (9) in his studies during the winter months also was able to distinguish 3 classes that gave approximately a 1:2:1 ratio. During the summer months, reactions were so modified that individuals, heterozygous or homozygous for resistance, could not be distinguished. In his studies Dietz (5) found susceptibility dominant.

SUMMARY

The reactions of 85 varieties of barley in the seedling stage in greenhouse studies to 2 new physiologic races of *Erysiphe graminis hordei* are given. These 2 races are designated as physiologic races 6 and 7. To the list of 4 differential barley varieties used by Mains and Dietz (15), a fifth variety, Heil's Hanna 3 C. I. 682, was added.

Five wild species of Hordeum were highly resistant to physiologic race 6 in tests conducted in winter in the greenhouse.

Seedling barley plants, in winter and spring, have shown no marked changes in reaction at these two seasons to either race 6 or race 7. Certain varieties are slightly more resistant in spring.

Tests of varieties in the adult stage in greenhouse studies, have shown that such barley plants, in spring, are more resistant to mildew than seedling plants of the same varieties. Adult plants in winter are fully as susceptible as seedling plants of the same varieties.

Studies of F_2 and F_3 of 3 crosses between resistant and susceptible plants indicate that resistance or susceptibility to physiologic race 6 of barley mildew in the seedling stage is inherited in definite Mendelian proportions. (1) In the cross, Svansota M786 × Hanna C. I. 906, a single pair of factors is involved. Resistance is incompletely dominant. (2) In the cross, Featherston C. I. 1118 × Goldfoil C. I. 928, a single pair of factors is involved. Resistance is incompletely dominant. The reactions of heterozygous individuals were altered by environmental factors. Independent inheritance of the factor pairs for resistance versus susceptibility, and 2-row spikes versus 6-row spikes is indicated. (3) In the cross, Arequipa C. I. 1256 × Horsford C. I. 610, a single pair of factors is involved. Resistance is dominant. Independent inheritance of the factor pairs for resistance versus susceptibility and hoods versus awns is suggested.

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STUDIES ON REPRESENTATIVE STRAINS OF TOBACCO-MOSAIC VIRUS[1]

JAMES H. JENSEN

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INTRODUCTION

Several investigators have described symptoms produced by one or more strains of tobacco-mosaic virus (1, 2, 3, 5, 6), but no effort has been made to define or to describe the range of symptoms produced by any considerable number of strains in different species. Demonstration and description of the extensive range of symptoms obtainable by infection with various strains of tobacco-mosaic virus will, it is believed, aid in arriving at a better understanding of the relationships between virus strains.

Fifty-five distinct strains of tobacco-mosaic virus were studied. These were Johnson's tobacco virus 1 (5) and 54 strains derived directly or indirectly from this virus. Symptoms produced by each strain varied in some respect from those produced by any of the other strains. The purpose of this report is to show the extent of the symptom range exhibited by these strains of tobacco-mosaic virus and to report on the differences in infectivity and resistance to heat shown by the strains of virus.

MATERIALS AND METHODS

Fifty-one of the 54 strains were obtained by making single pin-puncture inoculations to healthy tobacco plants from bright yellow spots on tobacco, tomato, or *Nicotiana sylvestris* Spegaz. and Comes plants infected with tobacco-mosaic virus (3, 4). The other 3 were obtained from various sources. One was the masked-symptom strain isolated by Holmes (2). The second appeared in one of 10 tobacco plants that had been inoculated with virus from a single necrotic lesion on a leaf of *N. langsdorffii* Weinm.[2] previously inoculated with tobacco-mosaic virus. The third was isolated in the following manner: A diluted sample (1:50) of tobacco-mosaic virus was rubbed over 3 large expanded leaves on each of 123 *N. sylvestris* plants. Within a few days many chlorotic lesions and, in addition, a small number of circular necrotic lesions appeared on the 369 inoculated leaves. In the hope that a virus capable of producing only necrotic lesions on leaves of *N. sylvestris* would be isolated, several of the necrotic lesions were cut out and transferred, by the glass-spatula method (3), to leaves of healthy *N. sylves-*

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[2] This plant referred to in previous publications (3, 4) as *N. langsdorffii* Schrank.

tris plants. Many yellow and a few necrotic primary lesions appeared on the inoculated leaves. Virus samples from single necrotic lesions were again transferred as before and both yellow and necrotic lesions were produced. After making a series of 5 such transfers, the virus causing only necrotic lesions on *N. sylvestris* was obtained. It was similar to the green-mosaic virus isolated by Kunkel (6) and by Smith (7) from plants affected by aucuba mosaic.

For the most part, the host plants used were *Nicotiana tabacum* L. var. Turkish, *N. sylvestris,* and *N. glutinosa* L. In a few instances plants of *Lycopersicon esculentum* Mill. also were used. Data on symptoms were based on observations of a succession of inoculated plants studied during a period of several years. All plants were grown in 4-inch or 6-inch unglazed clay pots or in shallow wooden flats. The greenhouse temperature varied from about 68° F. to about 90° F., with an average temperature of about 72° F.

The rubbing method of inoculation was used in all routine transfers of virus. The efficacy of this method was augmented by the use of finely ground carborundum (No. 320) for the transfer of virus strains having low infectivity. Other special methods of inoculation used have been described previously (3).

All thermal inactivation and infectivity studies were based upon inoculations made with freshly expressed juice. Except in the case of the masked-symptom strain virus, samples were obtained only from leaves showing symptoms. The upper leaves of plants infected with the masked-symptom strain were used. The tissue was ground in a sterile food chopper and the juice expressed by pressing the pulp by hand in a cheesecloth bag. Temperature exposures of juice from diseased plants were made by placing small test tubes (1×10 cm.) containing 3 cc. of juice in a large insulated water bath warmed by a hand-regulated electric heater, and constantly agitated by means of an electrically driven stirring rod. The water bath was held within 0.5° C. of the desired temperature.

CHARACTERISTIC SYMPTOMS PRODUCED BY 12 REPRESENTATIVE VIRUS STRAINS

Twelve strains of tobacco-mosaic virus were selected for detailed study. The symptoms produced by these 12 strains are representative of those caused by the 55 strains studied. Table 1 presents a brief description of the symptoms produced on 3 host species, *Nicotiana tabacum* var. Turkish, *N. sylvestris,* and *N. glutinosa.* The following statements indicate the outstanding characteristics of each of the strains, as well as the characteristics that distinguish the various strains from ordinary tobacco-mosaic virus.

Green-mottling distorting-type strain (Johnson's tobacco virus 1 (5)): Symptoms of this virus, commonly known as ordinary tobacco-mosaic virus,

TABLE 1.—*A brief description of the symptoms produced by 12 strains of tobacco-mosaic virus on 3 host plants*

Virus strain	Tobacco		*Nicotiana sylvestris*		*Nicotiana glutinosa*
	Primary lesions	Systemic infection symptoms	Primary lesions	Systemic infection symptoms	Primary lesions
Ordinary tobacco mosaic	Faint yellow	Green mottling	Faint yellow	Green mottling	Large[a]
101	Bright yellow	Erect leaf and yellow mottling	Necrotic	Systemic necrosis	Ordinary[b]
108	Bright yellow	Yellow mottling	Necrotic	None	Ordinary
501	Faint yellow	Green mottling	Necrotic	None	Ordinary
302	Bright yellow	Yellow mottling	Yellow	Yellow mottling	Ordinary
111	Yellow	Yellow mottling	Large yellow	Yellow spotting	Ordinary
9	Faint yellow	Light yellow spotting	Necrotic	None	Ordinary
104	Small yellow	Yellow spotting	Small yellow	Yellow spotting	Minute
502	Faint yellow	Mild green mottling	Faint yellow	Mild green mottling	Ordinary
Masked-symptom	Invisible	Invisible	Invisible	Invisible	Small[c]
3	Yellow	None	Yellow	None	Ordinary
14	Necrotic	None	Necrotic	None	Ordinary

[a] 1 to 2 mm. in diameter 5 days after inoculation.

[b] Like lesions produced by tobacco-mosaic virus.

[c] Slightly smaller than lesions produced by tobacco-mosaic virus.

have been completely described many times, and no need is felt for further discussion of them here. All other strains mentioned in this report were derived directly or indirectly from this virus strain. Figure 2, A, shows symptoms of this virus on tobacco.

Yellow-mottling distorting-type strain: In tobacco this virus strain, listed as No. 101, causes a yellow mosaic, which reaches the growing point and produces mottling and distortion of young leaves at the tip of the plant. From 1 to 2 days after the clearing-of-veins symptom is first observed, 1 or sometimes 2 of the leaves that show this symptom assume a marked upright or erect position in which they remain for 1 or more days. Figure 1 is from a photograph of a plant showing this peculiar response. This strain kills young plants of *Nicotiana sylvestris.*

Yellow-mottling aucuba-type strain: This virus, designated as No. 108, produces symptoms essentially like those described for aucuba mosaic (7). It causes yellow mottling in tobacco and necrotic primary lesions in *Nicotiana sylvestris.* Figure 2, C, shows the symptoms produced on tobacco.

Photograph by J. A. Carlile

FIG. 1. Symptoms of strain No. 101 on tobacco. The uppermost leaf in the photograph shows the upright position assumed by one of the vein-clearing leaves 5 days after inoculation. This leaf assumed its normal position 3 days after the photograph was taken.

Green-mottling aucuba-type strain: Virus No. 501 produces symptoms essentially like those described by Kunkel (6) and Caldwell (1) for the green aucuba-mosaic virus. It causes green mottling in tobacco and necrotic primary lesions in *Nicotiana sylvestris.*

Yellow mottling-type strain: Virus No. 302 produces a typical yellow mosaic, but is slightly less invasive than the viruses causing the yellow mosaics mentioned above. It produces yellow mottling in both tobacco and *Nicotiana sylvestris.*

Yellow spotting-type strain: This virus, designated as No. 111, is moderately slow-moving in tobacco plants, causing large yellow spots with centers of deep green instead of mottling symptoms. It differs sharply from the other virus strains in that it never produces vein-clearing symptoms in tobacco (Fig. 2, D).

Light green spotting-type strain: This virus, listed as strain No. 9, causes light green spotting instead of green-mottling symptoms in tobacco. Several weeks after inoculation, inconspicuous brownish necrotic spots appear on some of the older leaves.

Photograph by J. A. Carlile

FIG. 2. Symptoms produced on tobacco by 4 strains of tobacco-mosaic virus. A. Ordinary tobacco-mosaic virus. B. Strain No. 104. C. Strain No. 108. D. Strain No. 111.

Mild yellow spotting-type strain: Virus No. 104 causes a systemic yellow spotting on tobacco, as is shown in figure 2, B, and can be readily distinguished from all other strains by the minute necrotic lesions it produces on *Nicotiana glutinosa.* Figure 6 illustrates 2 leaves of *N. glutinosa,* one inoculated with ordinary tobacco-mosaic virus and the other with strain No. 104.

Non-distorting green-mottling strain: Virus strain No. 502 causes a mild green mottling of tobacco, the symptoms of which are identical with or simi-

lar to those described by Holmes (2) under the name of "mottling strain."

Masked-symptom strain (Holmes(2)): Ordinarily sets of tobacco plants inoculated with this virus strain can not be distinguished from sets of non-inoculated plants. It causes systemic infection without the production of visible symptoms.

Photograph by J. A. Carlile

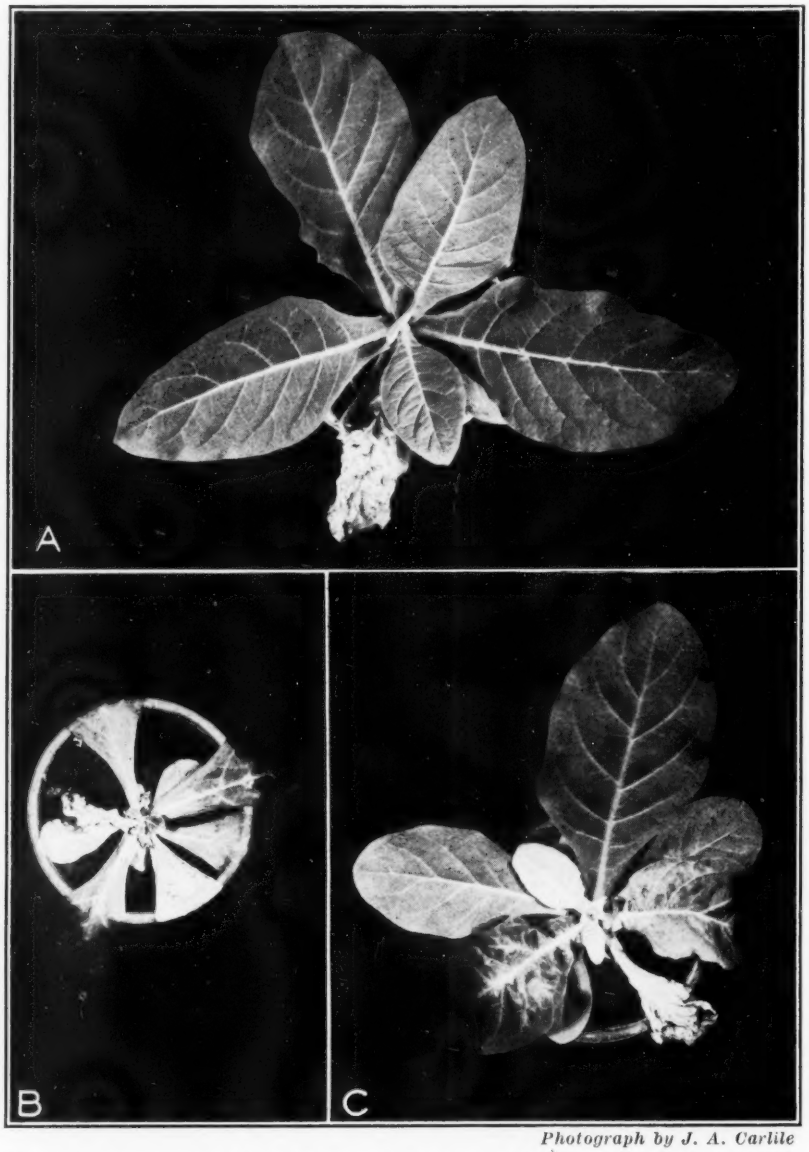
FIG. 3. Symptoms of strain No. 3 on tobacco. Twelve days after inoculation: no symptoms other than yellow primary lesions have appeared.

Slow-moving yellow-spotting-type strain: Virus No. 3 is very slow moving, causes pale yellow spots on tobacco leaves, as shown in figure 3, never causes mottling, and is of low infectivity. The yellow primary lesions tend to enlarge along the midveins. In tobacco, yellow oak-leaf patterns occasionally appear on leaves near the tips of infected plants.

Necrotic-type virus strain: This virus strain, designated as No. 14, produces necrotic primary lesions on tobacco, as shown in figure 4, A and B, never becomes systemic, and is of low infectivity. In tomato and tobacco the necrosis sometimes spreads down the petiole of infected leaves and into the stem, causing the plants to fall over.

Photograph by J. A. Carlile

FIG. 4. Symptoms of strain No. 14 on tobacco. A. Twelve days after inoculation: no symptoms other than a necrotic primary lesion have appeared. B. Two months after inoculation: necrosis has extended down into the stem and the plant has fallen over. No symptoms have appeared in the tip leaves.



Photograph by J. A. Carlile

FIG. 5. Symptoms of 3 isolations on *Nicotiana sylvestris*. A. Strain No. 108 produced only necrotic primary lesions. The inoculated leaf subsequently collapsed and died. B. Strain No. 101 produced a severe chlorosis and the plant finally died. C. Strain No. 302 produced a yellow-mottling disease. The inoculated leaf died. All plants were of the same age and were inoculated at the same time.

RANGE OF SYMPTOMS

The 55 strains of tobacco-mosaic virus fall into 3 general classes when the symptoms appearing on tobacco are studied: (1) Those that produce a generalized systemic infection, as in the case of ordinary green and yellow mosaic strains. (2) Those that produce spotting without mottling. Viruses in this group cause vein-clearing only when heavy inoculations are made on several leaves at the same time. (3) Those in which systemic infection, in the usual sense, does not occur. Plants infected with viruses of this group occasionally show oak-leaf infection patterns on one or more of the lower leaves. The group includes slow-moving yellow-type and necrotic-type strains.

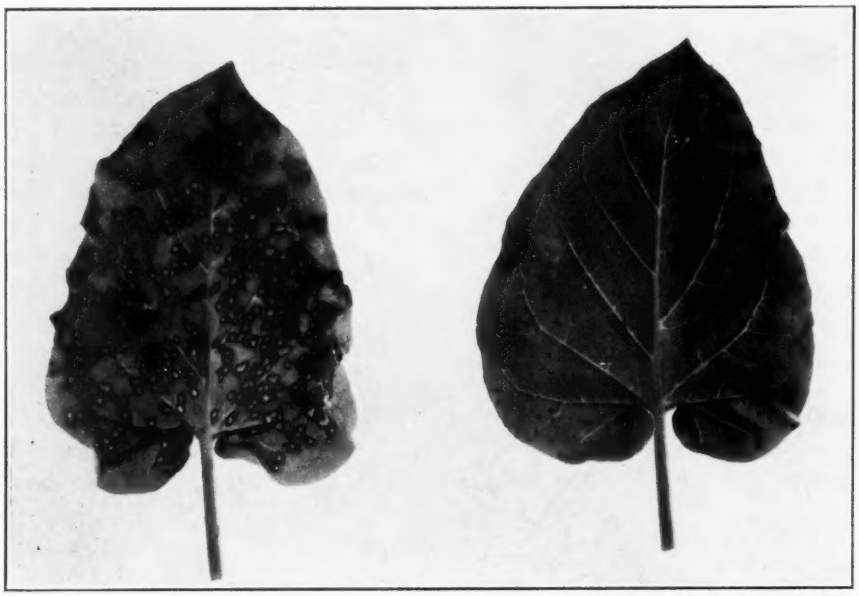
The reaction of *Nicotiana sylvestris* to certain strains is very striking, and for that reason this species was included as a test plant. In general, 3 types of primary lesions are produced: they may be yellow, similar to those obtained with ordinary tobacco-mosaic virus; they may be necrotic; or they may be invisible. Systemic infection in *N. sylvestris* produces green and yellow mottling diseases, systemic necrosis, and masked-symptom infection. Certain virus strains invariably kill young *N. sylvestris* plants. Usually such strains cause necrotic primary lesions. Figure 5 shows 3 types of reactions obtained in this plant.

The appearance of the necrotic primary lesions produced by tobacco-mosaic virus on leaves of *Nicotiana glutinosa* is well known. With 2 exceptions, all strains of virus that were tested produced necrotic lesions that appeared identical with those produced by ordinary tobacco-mosaic virus. The masked strain produced slightly smaller lesions than are caused by ordinary tobacco-mosaic virus, whereas strain No. 104 produced lesions that were much smaller, as shown in figure 6. These minute lesions required a longer time for development than do the lesions caused by tobacco-mosaic virus.

INFECTIVITY EXPERIMENTS

Throughout the course of these investigations it was noted that virus strains show great variations in the ease with which they are transmitted. A number of strains were found to be as easily transmitted as ordinary tobacco-mosaic virus, the high infectivity of which is well known, whereas others were considerably less easily transferred, and still others were transmitted to healthy plants only with difficulty. A high percentage of infections was obtained with the latter strains only through the use of powdered carborundum.

In an attempt to present the range in transmissibility on a numerical basis, several series of experiments were designed in which 2 types of inoculation and 2 species of host plants were used. In the first series of experi-



Photograph by J. A. Carlile

FIG. 6. Necrotic primary lesions on *Nicotiana glutinosa*. Left: Ordinary lesions produced by tobacco-mosaic virus. Right: Minute lesions produced by strain No. 104. Both leaves were of the same age and inoculated on the same day.

ments single pin-puncture inoculations were made from leaves of diseased plants to a small leaf on each of 50 healthy tobacco plants. The number of

TABLE 2.—*Number of infected plants obtained by making single pin-puncture inoculations into sets of 50 young tobacco plants with each of the 12 tobacco-mosaic virus strains studied*

Virus strain	Test 1	Test 2	Test 3	Test 4	Total
Ordinary	19	25	23	28	95
101	19	15	16	18	68
108	8	9	12	10	39
501	14	20	11	10	55
302	6	8	2	2	18
111	0	0	0	0	0[a]
9	8	12	13	14	47
104	28	25	21	22	96
502	14	16	17	15	62
Masked-symptom	16	13	14	14	57
3	0	0	0	0	0[a]
14	0	0	0	0	0[a]

[a] In additional tests single pin-puncture inoculations were made to 700 young healthy tobacco plants with each of virus strains 111, 3, and 14. From this number of inoculations 6 plants were infected with strain 111, 1 with strain 3, and 2 with strain 14.

inoculated plants that became diseased varied from almost 50 per cent, in the case of 2 mottling-type strains, to less than 1 per cent, in the case of a slow-moving yellow-spot virus strain. Table 2 shows the number of diseased plants obtained in 4 different tests and the total number of infections obtained in all 4 tests.

A second series of experiments was set up in which various dilutions of the virus strains were rubbed over leaves of *Nicotiana glutinosa* and the resulting number of necrotic primary lesions were recorded. The Latin-square method proposed by Youden and Beale (8) was utilized. Experiments were arranged so that the same dilutions of 5 virus strains were compared in each set of plants. Each virus strain was represented on each plant once and on each leaf position once in every test. The tests were repeated a number of times, and in each repetition a different combination of 5 virus strains was used, the purpose being to test the individual virus strains against as many other virus strains as practicable.

The data from one test are shown in table 3; other tests gave similar results. In this and in other experiments the 2 slow-moving virus strains, Nos. 3 and 14, were not tested against the other virus strains because their low infectivity made it desirable to rub more than 5 test leaves and also to take extra precautions against possible contamination with other virus strains. The figures shown in the cases of strains Nos. 3 and 14 represent the average numbers of lesions that appeared on 25 inoculated leaves.

TABLE 3.—*Average numbers of lesions obtained per leaf of Nicotiana glutinosa rubbed with various dilutions of the tobacco-mosaic virus strains indicated*

Virus strain	Dilutions					
	1:1[a]	1:2	1:4	1:10	1:100	1:1000
Ordinary	290	270	190	91	47	10
101	139	92	84	62	27	5
108	63	53	36	29	13	3
501	63	67	37	28	7	1
302	69	52	56	22	7	1
111	0.4	0.8	0.2	0	0	0
9	71	73	56	25	15	1
104	162	106	106	55	28	2
502	103	113	79	66	34	7
Masked-symptom	121	138	84	69	28	11
3[b]	0.2	0	0	0	0	0
14[b]	0.2	0	0	0	0	0

[a] 1:1 represents undiluted juice.

[b] Figures for numbers of necrotic lesions obtained by inoculations with virus strains 3 and 14 are based upon 25 different leaf tests. No lesions were obtained with any diluted juice samples.

The experiments demonstrate that the strains of tobacco-mosaic virus vary widely in infectivity. Ordinary tobacco-mosaic virus is more infective than most of the other virus strains tested. It seems clear that the slow-moving strains are least infective.

THERMAL INACTIVATION EXPERIMENTS

One of the properties frequently used to characterize certain plant viruses is the thermal inactivation point. As is well known, the thermal inactivation point of tobacco-mosaic virus for a 10-minute exposure is about 90° C. Other plant viruses are inactivated at lower temperatures.

The variability in symptoms produced by various strains of tobacco-mosaic virus suggested the possibility that the virus strains might differ in their resistance to heat. Accordingly, viruses of the 12 strains, the symptoms of which have been described, were subjected to 10-minute exposures of 70°, 80°, 85°, and 89° C. In each experiment 10 viruses were divided into 2 sets of 5 viruses each, the 2 slow-moving strains, Nos. 3 and 14, being tested separately in order to minimize chances for accidental contamination. Juices containing each of the 5 virus strains in 1 test were exposed to the various temperatures simultaneously. Immediately after the temperature exposure, tubes containing the viruses were set in cold water to cool. Inoculations on leaves of *Nicotiana glutinosa* were carried out immediately after cooling. The Latin-square method of leaf arrangement was again used.

Table 4 shows the average numbers of lesions per inoculated leaf obtained with the strains. It is to be noted that lesions were obtained with virus of every strain exposed to a temperature of 80° C. for ten minutes, but that several virus strains apparently did not survive exposures to temperatures beyond that point. These results might be interpreted as indicating differences in thermal inactivation points. However, when results of infectivity experiments are studied it will be noted that the same strains that are inactivated at a low temperature also show low infectivity. The results of these two series of experiments, therefore, indicate that infectivity must be considered in interpreting thermal inactivation of a given virus.

DERIVED STRAINS

Plants inoculated with slow-moving virus strains occasionally show mottling or other characteristics of fast-moving strains. When subinoculations are made to healthy plants, fast-moving viruses are obtained. It is difficult to explain the presence of fast-moving strains in the tips of plants inoculated with slow-moving viruses in any other way than by assuming that the new strains arise from the viruses originally introduced into the plants.

Although the symptoms produced on young tobacco plants inoculated

TABLE 4.—*Average numbers of necrotic lesions obtained per leaf of Nicotiana glutinosa rubbed with samples of tobacco-mosaic virus strains heated for 10 minutes to the temperature indicated*

Virus strain	Temperature				
	Unheated	70° C.	80° C.	85° C.	89° C.
Ordinary	320	280	270	250	95
101	200	179	75	17	0.6
108	162	110	29	2	0.4
501	250	199	87	11	0.2
302	30	30	2	0.2	0
111	1	0.8	0.4	0	0
9	120	120	36	1	0.2
104	350	350	132	85	46
502	270	230	140	125	30
Masked-symptom	270	185	110	60	7
3	1	0.6	0.4	0	0
14	0.8	0.6	0.6	0	0

with virus from tip leaves of plants showing divergent symptoms seemed similar to those produced by ordinary systemic yellow and green mottling virus, nevertheless it appeared desirable to test these viruses on other host plants. Three of the isolations obtained from the tip leaves of tobacco plants inoculated with the slow-moving necrotic virus (strain No. 14) will be described. When the viruses were transferred to tobacco, 1 strain produced a green-mottling disease and the other 2 produced yellow-mottling diseases without any unusual features. When the viruses were transferred to tomatoes, one produced a green-mottling disease, another a yellow-mottling disease, and the third a severe yellow-mottling disease that was followed by a systemic necrosis which, when moderately young plants were infected, always resulted in death of the plants. The symptoms produced by these 3 derivative strains are presented in figure 7. Thus, from ordinary tobacco-mosaic virus a slow-moving necrotic-type strain was isolated, and from this slow-moving strain on tobacco a strain was derived that produced a fast-moving virus causing systemic necrosis in the tomato. Derivation of such highly destructive strains from relatively nondestructive strains may open the way for studies on the nature of variations in virus strains.

DISCUSSION

The outstanding characteristic symptoms of 12 strains of tobacco-mosaic virus on 3 species of host plants is presented in this report. The 12 strains produce symptoms that are representative of the range of symptoms of the 55 strains studied.

The variations in symptoms that were reported are characteristic of

Photograph by J. A. Carlile

FIG. 7. Symptoms produced on tomato by 3 strains derived from strain No. 14. A. Green-mottling strain. B. Yellow-mottling strain. C. Necrotic strain, which is lethal. All plants were of the same age and inoculated on the same day.

individual strains. Except in the case of the vein-clearing symptom produced when several leaves were heavily inoculated with spotting viruses, the symptoms produced by a given virus sample were always the same regardless of the dilution of the inoculum or the amount of leaf surface inoculated. No information was gained as to the underlying differences between the virus particles responsible for the variations in symptoms. It can not be said, for instance, whether inability of the slow-moving strains to move rapidly or their low infectivity is due to excessive size, greater toxicity, slower multiplication, or to some other factor. A general correlation was found to exist between rapidity of virus movement and infectivity. All slow-moving strains were difficult to transmit, whereas strains characterized by rapid movement were readily transmissible.

The appearance of mottling viruses in the tip leaves of plants inoculated with slow-moving viruses might seem to indicate that the slow-moving viruses are highly unstable. It will be recalled, however, that most of the strains used in this study have been isolated from plants inoculated with the ordinary tobacco virus, a fact which indicated that variations arise in the rapid-moving viruses also. Slow-moving viruses arising in plants inoculated with rapid-moving viruses have been found only in bright yellow spots. Their inability to move probably prevents their further distribution in the plant tissue. However, when a rapid-moving virus arises from a slow-moving virus, its ability to move into new tissue makes its appearance all the more striking. The derivation of necrotic virus strains from an ordinary widely distributed virus strain presents interesting possibilities. Further work in deriving strains experimentally in this manner may lead to a discovery of the underlying reasons for the variations produced by different virus strains.

SUMMARY

Symptoms of 12 tobacco-mosaic virus strains, representative of the range of symptoms produced by 55 strains, are described on tobacco, *Nicotiana sylvestris*, and *N. glutinosa*. One strain, derived from a slow-moving, necrotic-type strain on tobacco, killed tomato plants. Two strains produced unusually small lesions on leaves of *N. glutinosa*. Single pin-puncture inoculations of some strains to young tobacco plants produced as high as 50 per cent infection, whereas other strains were transmitted in less than 1 per cent of the attempts. Other strains ranged between these two extremes. Infectivity trials using the local-lesion method gave similar results. All strains tested were found to withstand 10-minute exposures to a temperature of 80° C.

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STUDIES ON "DAMPING OFF" OF CULTIVATED MUSHROOMS AND ITS ASSOCIATION WITH FUSARIUM SPECIES[1]

F. C. Wood, B.A.[1]

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INTRODUCTION

During the course of routine visits to commercial mushroom growers in the south of England it was noticed that many of the beds showed a typical condition known to most of the growers as "damping off." The writer proposes to continue this nomenclature in the ensuing discussion, since it effectively describes the commoner type of condition and, moreover, it is already in use among mushroom growers.

The 3 following characteristic conditions, constantly observed during preliminary investigations on "damping off," were: 1. The beds were full of spawn run, and in many cases the spawn also had run into the casing soil, but little or no mushroom production had taken place.

2. Mushrooms had formed, even to the extent of a good first flush. The crop rapidly degenerated into small, withered, brown, and undeveloped mushrooms. Rotting generally did not take place, the stunted mushrooms remaining indefinitely on the beds in a mummified condition.

3. The crop appeared to be normal and, on occasions, heavy. Sooner or later a big proportion of the mushrooms were found to be dry and pithy inside the stipe, of a rubbery texture, and, generally, the interior of the stipe was quite brown. These mushrooms were of no market value. In the case of the brown variety of mushroom the pilei were burnished and shining in appearance, giving the impression of having been polished, while the normal mushroom showed a matt brown pileus.

No clear demarcation exists between the 3 types of condition, all of which often were found simultaneously on a bed.

In each of the above cited cases it was found that other beds in the same house, under the same growing conditions, and prepared from the same compost, were producing perfect mushrooms. It also often happened that the trouble was localized to a patch, in and around which perfectly healthy mushrooms were formed.

[1] The writer is particularly grateful to Dr. H. W. Wollenweber of the Biologische Reichsanstalt, Berlin-Dahlem, for his unfailing kindness and courtesy in the indentification of the many species of Fusarium that were isolated during the course of these investigations. He also owes a debt of gratitude to Mr. G. R. Rettew and the Chester County [Pennsylvania] Mushroom Laboratories for their continued help and encouragement, and to the many mushroom growers and fellow mycologists who, from time to time, have so kindly given facilities.

These factors combined with careful observation and attention to cultural details gradually eliminated the possibility of the trouble being due either to pest attack or to faulty watering, bad ventilation, etc.

It also was noticed that "damping off" often would spread rapidly through a bed, and it, therefore, was assumed that a fungus was responsible. With this idea in mind samples from mushroom beds from various commercial growers were examined systematically, special attention being given to the casing soil, since this was generally the only part of the bed that was unsterilized.[2]

Finally, from the material forwarded from Kings Lynn, a *Fusarium* species was obtained and a preliminary inoculation[3] experiment suggested that the real cause of "damping off" of cultivated mushrooms had been found.

Working on this hypothesis, a systematic examination of as many cases of "damping off" as were available was begun and, with surprisingly few exceptions, a Fusarium species was found in the casing soil in close proximity to the affected mushrooms.

By far the commonest species were *Fusarium martii* (App. and Wr.) and *Fusarium oxysporum* Schl., and these two species were taken for experimental purposes.

Other species isolated were *F. culmorum* (W. G. Sm.) Sacc., *F. flocciferum* Cda., and several others not yet identified.

TECHNIQUE

In all the material examined the following methods were used for determination and isolation of the fungi concerned.

Culture plates were prepared, using the following medium: Cane sugar, 25.0 g.; sodium nitrate, 2.0 g.; potassium chloride, 0.5 g.; potassium phosphate, (KH_2PO_4) 1.0 g.; magnesium sulphate, 0.5 g.; ferrous sulphate, a trace; agar agar, 30.0 g.; sodium tauroglycocholate, 1.0 g.; distilled water, 1000 cc.

It was found that this medium did not tend to encourage bacterial growth; therefore, the presence or absence of fungus species in the soil surrounding the bases of the stipes of "damped off" mushrooms was determined by plating out soil particles.

If *Fusarium* spp. were present, they grew out from the soil particles and speedily outgrew any bacterial contamination. (Fig. 1, A.) Pure cultures

[2] During the preparation of the compost temperatures of 140–160° F. are often reached. These are far above the thermal death point of *Fusarium* sp. The greater part of the spawn now used is pure culture spawn.

[3] Wood, F. C. "A New Disease of Cultivated Mushrooms." Gard. Chron. XCVII. 243. 1935.

then were obtained by subculturing repeatedly on the following medium: 100 g. soil mixed with 100 cc. water and autoclaved for 30 min. at 10 lbs. pressure. To the filtrate was added 5 per cent malt extract and 2 per cent agar agar.

As regards the inoculation experiments it was obvious from the beginning of the investigation that the following disadvantages would have to be reckoned with: (a) The length of time elapsing between spawning and production is two months. This makes an investigation liable to error, since it necessitates careful control of conditions over a long period. (b) The compost used for growing the spawn is not sterile and cannot be sterilized easily and effectively. (c) The casing soil must be sterilized. (d) The spawn must be uncontaminated. (e) Rigid pest control must be maintained.

The commonest pests to be reckoned with are species of Hypogastrura; species of Tyroglyphus; Sciarid flies; and Phorid flies.

After many unsuccessful efforts with inoculation experiments in which results were negatived by infestation by Phorid fly larvae,[4] the following procedure was adopted: Glass pneumatic troughs, 10 cm. deep and 25 cm. wide, were used. Into these was pressed moist pure culture spawn to a depth of about 6 cms. After allowing a few days to elapse, in order to allow the spawn to run throughout the mass again, the casing soil was applied.

This was good quality clay loam, previously sterilized by heating for 20 min., under steam pressure, to a temp. of 80° C. When cool the soil was broken up and applied to a depth of $\frac{3}{4}$–1 in.

The materials in use, therefore, were sterile, since the spawn was broken out directly from the bottle in which it was grown and the soil was sterilized.

This method also eliminated trouble from springtails or mites in the compost. Sciarid and Phorid flies were prevented from ovipositing by covering the pneumatic trough with a layer of coarse muslin held in position by a rubber band. This method of mushroom growing was found to answer excellently under ordinary laboratory conditions and to produce mushrooms in about 3 weeks.

Inoculations were carried out by spraying the casing soil with a spore suspension of the Fusarium species used.

DAMPING OFF OF CULTIVATED MUSHROOMS CAUSED BY FUSARIUM OXYSPORUM SCHL. AND FUSARIUM MARTII APP. AND WR.

Symptoms and General Description

In the majority of cases investigated the trouble was noticed by the grower either towards the end of the crop or following a period of heavy picking. Both white and brown varieties of mushrooms have been found to

[4] The species was identified by Dr. Barnes of Rothamsted as *Aphiochaeta halterata*.

be affected, but usually *Fusarium oxysporum* seems to affect the brown variety of mushroom more than the white.

Typically the effect is that mushrooms make their appearance and for a time seem perfectly normal even to maturity. When picked, however, they

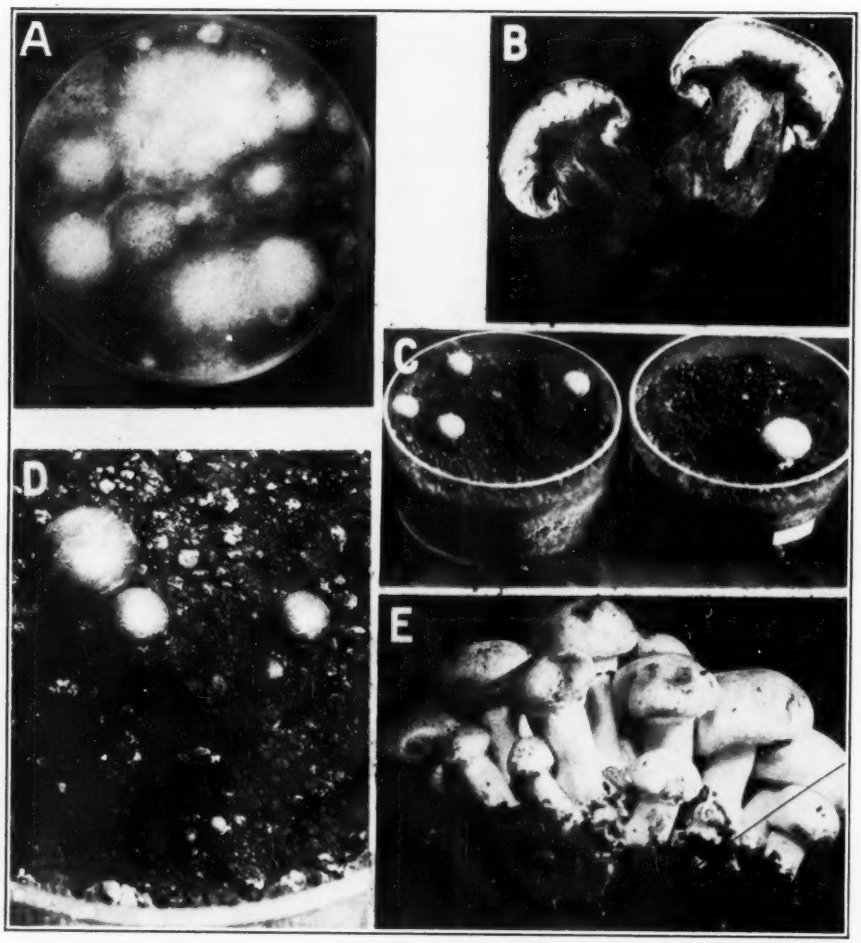

FIG. 1. A. Soil plate showing both *Fusarium oxysporum* and *F. martii*. The large white masses are the mycelium of the former, while the colony on the top left-hand edge of the Petri dish is the mycelium of the latter. ×1. B. Longitudinally cut mushrooms showing brown staining caused by *F. oxysporum*. ×1. C. Method of growing mushrooms in pneumatic trough. Control dish, left; inoculated dish, right. ×¼. D. Effects of inoculating casing soil with a spore suspension of *F. oxysporum*. The upper three mushrooms are normal brown type. Below, on the right, is a smaller mushroom showing the glossy dark brown pileus. Towards the lower edge of the dish are normal and affected buttons side by side. ×½. E. Break of white mushrooms from first flush showing effect of *F. oxysporum* in casing soil. ×⅔.

show a pithy, withered nature and the interior of the stipe is generally brown, the discoloration showing a gradation in intensity from the base of the stipe to the pileus. (Fig. 1, B). Mushrooms of this type appear in patches among and in close proximity to healthy breaks. Such mushrooms are, of course, valueless for market. If they are picked off, healthy ones may be produced for a time. Eventually, however, the trouble reappears—generally having spread down the beds—and now there is a distinct external difference from the healthy crop. The pileus presents a burnished appearance, totally distinct from the usual matt brown of the healthy mushroom, and has a polished surface. The shade of color is darker than that of the normal mushroom. As before, this stage persists for some time, alternating with healthy mushroom production (Fig. 1, D).

The third stage shows a diminution in size from the normal and, furthermore, a characteristic lopsidedness is noticeable. The stipe being eccentrically placed instead of centrally in relation to the pileus.

This leads to the last stage in which the mushroom never grows beyond the button stage in which stage it becomes mummified and persists on the bed for as long as a month without rotting. (Figs. 2, E and F).

In one or two instances where a heavy infection of Fusarium in the casing soil was found, the crop was small and stunted from the outset and never gave promise of being a commercial proposition.

In the case of the white variety of mushroom only two instances of *Fusarium oxysporum* effect have been investigated. In both cases the crop appeared in breaks as in figure 1, E, characterized by very long stems, small pilei, discolored flesh and extremely rapid rotting and decomposition of the breaks.

The effect of *Fusarium martii* infection of the casing soil on mushroom production is much the same, and the above description, with but little modification, will serve. In the case of *F. martii* it seems that the white variety of mushrooms is far more readily affected than the brown and, up to the present time, no case of "damping off" of brown mushrooms has been found in which *F. martii* was responsible.

Inoculation Experiments with *Fusarium oxysporum* on Brown and White Varieties of Mushrooms

A. *Brown Variety.*—On July 8 two culture dishes were set up as previously described using brown pure culture spawn and a fairly heavy clay loam casing soil. One dish was used as a control throughout. The other was inoculated by spraying the casing soil with a spore suspension of *Fusarium oxysporum* in distilled water on July 12.

Both dishes were kept under normal laboratory conditions and were watered with sterile distilled water as the spawn ran up into the casing soil.

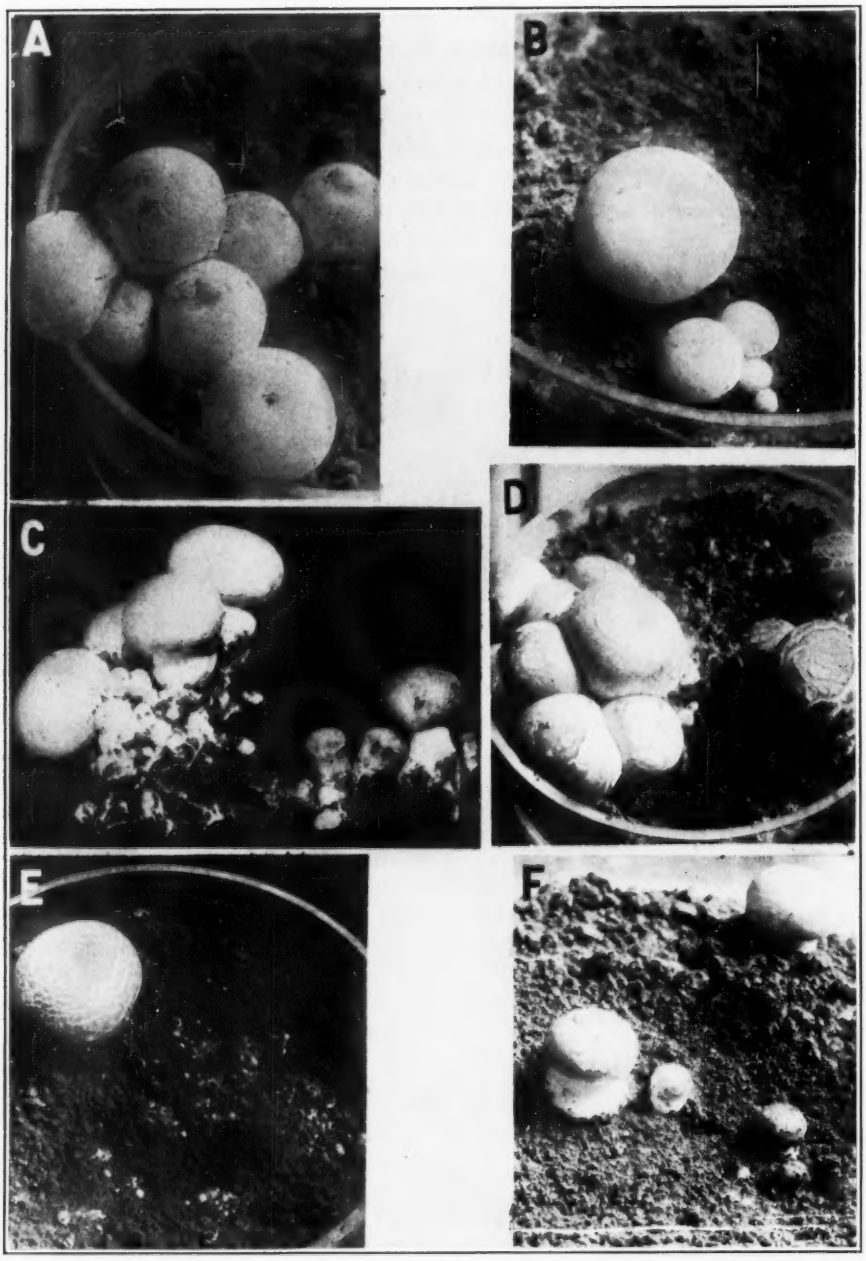

FIG. 2. A and B. *F. oxysporum* and its effect on white mushrooms. A. Control dish. B. Inoculated dish. $\times \frac{2}{3}$. C. *F. martii* and its effect on white mushrooms shown on right; control mushrooms on left. $\times \frac{2}{3}$. D. Brown control dish, *F. martii*. $\times \frac{1}{2}$. E. Inoculation experiment showing healthy mushroom, stunted and mummified "buttons," and patches of *F. oxysporum* showing in the soil. $\times \frac{1}{2}$. F. Inoculated dish. Note shrivelled buttons and tiny pips in the foreground. Compare mushrooms with the one in the background, in which, note absence of bulbous stipe, etc. $\times \frac{1}{2}$.

On July 26, just over a fortnight from the commencement of the experiment, "pipping up" of the spawn was taking place in the casing soil of the inoculated dish.

The first mushrooms were picked on August 6 (Fig. 1, C, D).

On October 10 (3 months from the beginning of the experiment) soil samples were taken from both the control and inoculated dishes and plates were made as described previously.

In the case of the control dish no Fusarium growth was obtained, the plate showing only bacterial contaminations and *Penicillium* sp.

TABLE 1.—*Yield in grams of brown and white varieties of mushrooms grown on heavy clay loam casing soil in culture dishes. One was a control series, the other a series of cultures inoculated with a spore suspension of Fusarium oxysporum*

Date	Yield from control cultures	Yield from inoculated cultures
A. Brown mushrooms		
Aug. 7	105	105.0[a]
" 16	.....	29.0[b]
" 19	64	.....
" 29	.....	0.5[c]
Sept. 2	26	.....
" 16	20	23.0
" 19	.....	.....
" 26	.....	26.5[d]
" 27	12	8.5
Oct. 7	3	13.0
Totals	230	205.5
B. White mushrooms		
April 6	38.3	.....
" 7	56.0	.....
" 9	23.6	.....
" 18	Fresh sterile soil added ½ in. deep	Fresh sterile soil added ½ in. deep
" 22	17.2	One small button visible
" 30	29.6	20.0
May 7	.....	5.5[e]
" 15	17.7	56.5
" 16	60.0	24.3
" 17	.....	30.4
Totals	254.4	136.7

[a] Many small shriveled pips present.

[b] Flesh not discolored, but leathery and of withered texture.

[c] All were stunted buttons; all brown and none exceeding ⅛ in. diam.

[d] Buttons now beginning to show "polished," dark brown appearance.

[e] Mushrooms very small, dwarfed and withered; interior of stipes stained brown.

In the case of the inoculated soil a very heavy growth of mycelium was obtained which eventually was proved to be *Fusarium oxysporum* Schl.

B. *White Variety.*—Inoculation experiments were carried out along the lines indicated, using white pure culture spawn. The experiment was set up on Feb. 20, 1936, and one dish was inoculated by spraying the sterile casing soil with a spore suspension of *Fusarium oxysporum.*

The first production was seen on the control dish on March 26 (Fig 2, A and B).

Comparing results it is seen that the ratio $\frac{\text{Control production}}{\text{Inoculation production}} = \frac{1}{.88}$ in the case of brown mushrooms and $\frac{1}{.54}$ in the case of white mushrooms. These results agree with those observed among commercial growers.

In all cases of *Fusarium oxysporum* infection of the casing soil and brown mushrooms (provided the crop develops under reasonably good conditions), the trouble seldom becomes apparent until the conclusion of crop growth, when the virility of the spawn is beginning to weaken. In cases where *F. oxysporum* infection of the soil is present with white mushrooms the crop generally is doomed to failure at the outset. It would appear, therefore, that the brown type of cultivated mushroom shows resistance to "damping off" by *F. oxysporum.*

Inoculation Experiments with *F. martii* on Brown and White Varieties of Mushrooms

A. *Brown Variety.*—This experiment was set up on Feb. 20, 1936. One dish was inoculated with a spore suspension of *F. martii* on March 19 (Table 2).

B. *White Variety.*—Inoculation experiments also were carried out, using the other commonly isolated Fusarium species from cases of "damping off," *viz., F. martii* App. and Wr.

The production ratio of $\frac{\text{Control dish production}}{\text{Inoculated dish production}}$ in the case of the brown variety of mushrooms is $\frac{1}{.93}$ and in that of the white is $\frac{1}{.48}$.

This is consistent with observed results in mushroom houses, as *F. martii* is exceptionally bad in beds of white mushrooms, while it seldom causes trouble in mushroom houses spawned with brown spawn.

DISCUSSION

From the results of the inoculation experiments it will be seen that there is a discrepancy in the weight of mushrooms produced in noncontaminated soil and that obtained from soil inoculated with *Fusarium* spp. This difference in weight is not strikingly great but it should be remembered that

TABLE 2.—*Yield, in grams, of brown and white varieties of mushrooms grown on casing soil in culture dishes. One was a control series, the other a series of cultures inoculated with a spore suspension of Fusarium martii*

Date	Yield from control cultures	Yield from inoculated cultures
A. Brown mushrooms		
March 21	31.7	
" 30	.....	3 mushrooms and a few small buttons
April 2	.....	20.6[b]
" 4	.....	22.5
" 6	120.0[a]	23.0, a few pips, withered
" 7	13.0	.....
" 9	.....	18.0, mushrooms perfect
" 18	Fresh sterile soil added	Fresh soil added
" 29	17.5	56.0
" 30	14.0	35.8
May 1	29.0	.....
" 15	25.0	39.7
" 16	.....	19.1
Totals	250.2	234.7
B. White mushrooms		
Aug. 12	7.5	.....
" 13	5.0	.....
" 20	7.0	.....
" 21	36.0	.....
" 24	1.0	8.75
" 30	6.5	.....
" 31	2.5[c]	10.5
Sept. 9	9.5	21.0
" 20	8.0	.....
Totals	83.0	40.25

[a] See figure 2, D and F.
[b] Mushrooms were perfect.
[c] See figure 2, C.

nothing like normal production is possible under the growing conditions that were perforce adopted.[5]

The supply of food material represented by the moist spawn is very small as compared with the fresh compost available in a newly prepared mushroom bed. Therefore, the production of the control dish is very far

[5] The market value also must be considered, since, although the mushrooms with withered stems or with "brassy" caps weigh well, they are, of course, utterly worthless from a commercial standpoint.

short of being normal. This is shown very well in the weight and size of the mushrooms themselves, which, at maturity, were probably only a third of the weight and size of mushrooms produced on a proper mushroom bed, albeit they were perfectly healthy.

The purpose of the experiment was, however, to prove that the presence of *Fusarium* spp. in the casing soil would give rise to disease symptoms in mushrooms exactly similar to those obtained in cases of "damping off"; and this end was achieved, as hitherto described.

SUMMARY

The continual association of "damping off" of cultivated mushrooms and the presence of a species of Fusarium in the casing soil was noted.

Species associated with "damping off" symptoms were *Fusarium oxysporum, F. martii, F. culmorum, F. flocciferum, F. redolens, F. sambucinum, F. sambucinum* form 6.

The commonest species were *Fusarium oxysporum* and *F. martii.* These were, therefore, studied in detail.

Inoculation experiments, involving Fusarium-infested casing soil and its effect on mushroom production, are described.

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HISTOLOGICAL AND CYTOLOGICAL STUDIES OF ETHYL MERCURY PHOSPHATE POISONING IN CORN SEEDLINGS

JOHN E. SASS

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INTRODUCTION

The use of dusts in which the active ingredient is ethyl mercury phosphate produces a characteristic malformation of the seedlings of corn and other cereals. As described by Crozier,[1] the plumule and radicle of treated seeds fail to elongate rapidly, but they enlarge greatly in diameter. The malformation exhibits all gradations from nearly normal to a short, compact,

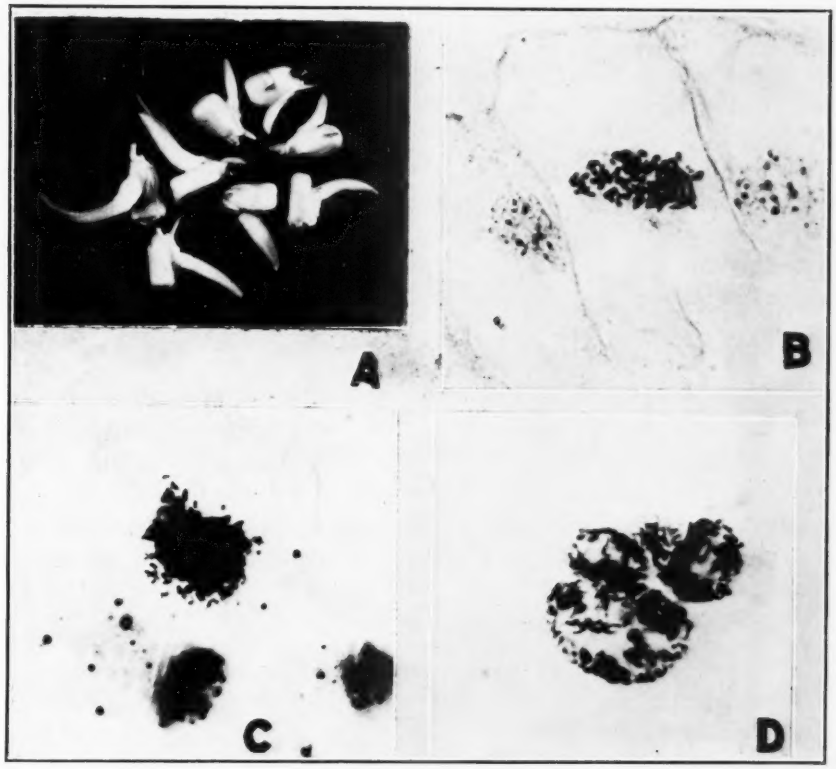

FIG. 1. A. Corn seedlings, two weeks after soaking seed corn in a 1:1500 solution of New Improved Ceresan. B–C. Metaphase figures of giant nuclei, showing large chromosome complement. The anaphase split can be seen in many chromosomes. ×700. D. Late prophase of the four nuclei in a single cell. The nuclei differ in size and chromosome complement. ×700.

[1] Crosier, W. Abnormal germination in dusted wheat. Phytopath. 24: 544–547. 1934.

fusiform condition. (Fig. 1, A.) The present study was undertaken to determine the anatomical and cellular changes associated with these malformations in seedlings of corn.

MATERIALS AND METHODS

A collection of seedlings grown after treatment by the usual dry dust method, was obtained from C. S. Reddy. Subsequent collections of material for sectioning were obtained from seedlings grown as follows: kernels of yellow-dent corn were soaked in a 1:1500 solution of New Improved Ceresan for 24 hours, drained, and germinated in moist chambers. All of the seedlings exhibited the malformation. Small sections were removed from the desired regions of seedlings and killed in the following fluid: 1 per cent chromic acid, 20 cc.; 1 per cent acetic acid, 75 cc.; formalin (40 per cent), 5 cc. An interval of 48 hours suffices for killing and hardening. No washing in water is required before dehydration in grades of acetone. The tissues of the coleoptile are sufficiently soft to section easily after the usual method of infiltration in paraffin. Pieces from the region of the scutellum, which include areas of brittle tissue, were dehydrated in a "butyl alcohol-acetone" series, essentially similar to the well known "ethyl alcohol-butyl alcohol" series. After imbedding by this process, sectioning is facilitated by soaking the mounted blocks of tissue in water at 35° C. for about 24 hours. The slides were stained with "Hemalum." Sections containing lignified cells were counter-stained with safranin.

For the study of cytological changes, root tips were used. Seedlings were grown in moist chambers, moistened with water, until the seminal roots reached a length of about 1 cm. The seedlings were then transferred to dishes containing blotting paper moistened with a 1:1000 solution of Ceresan. The growing roots, which curved down into the solution, developed pronounced terminal swelling and ceased apical elongation. These tips were cut off and prepared for sectioning. Sections were stained in iron-hematoxylin.

OBSERVATIONS

In corn seedlings grown from nontreated seed, the leaf primordia and apical meristem of the coleoptile have the structure characteristic of meristematic tissues. The cells are small, polygonal, compactly arranged, and of uniform size (Fig. 2, E). These cells are strictly uninucleate, and the nuclei are of uniform size. In older leaf primordia the cells are larger, more vacuolate, and begin to show evidence of differentiation into tissues, but the uninucleate condition is obvious. The surface outlines of the young leaves are smooth and nonlobate (Fig. 2, B–E).

Seedlings from treated seeds exhibit varying degrees of distortion of

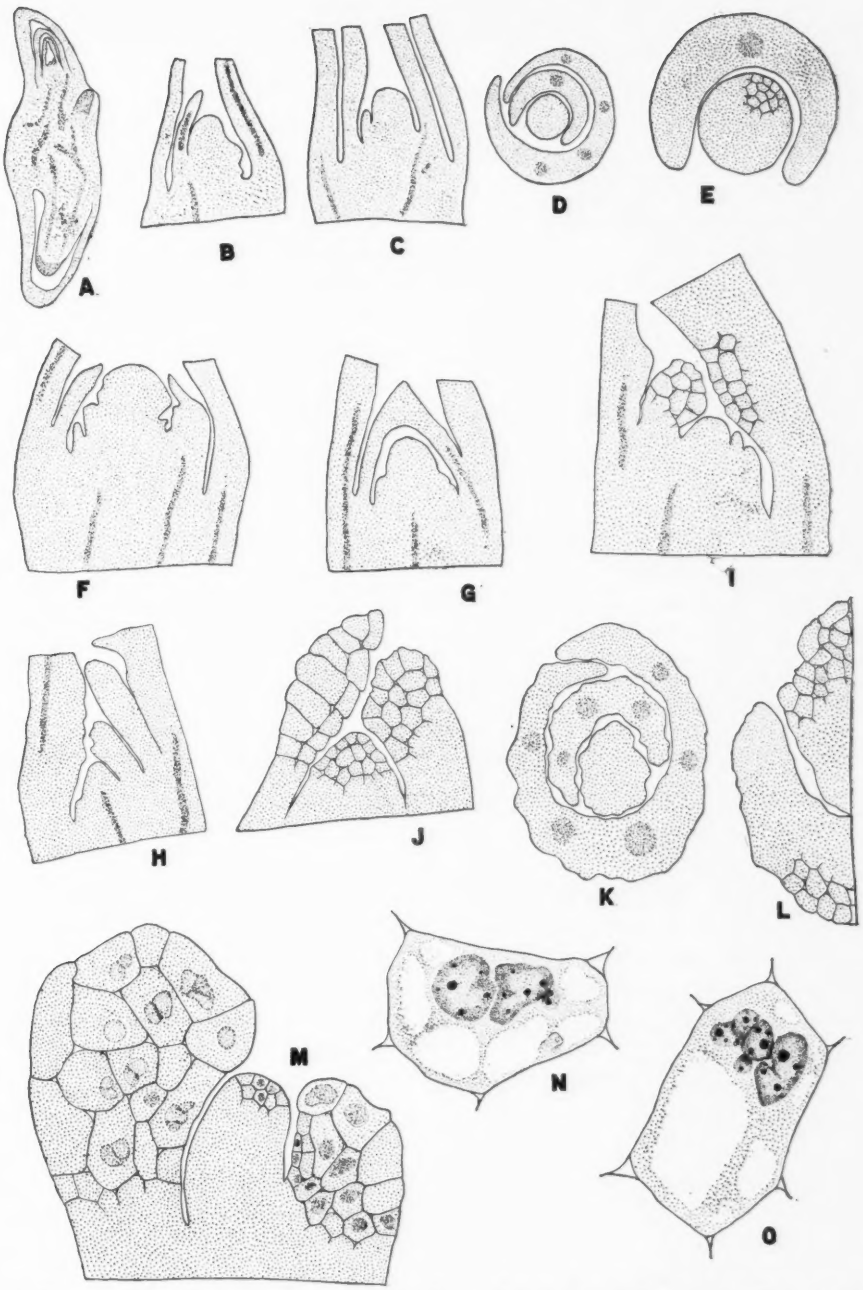

FIG. 2. A. Median section of normal embryo of corn, removed from scutellum, showing the sheathed apical meristems. B–C. Growing point of normal stem, showing leaf primordia. D. Transverse section near tip of normal growing point. E. Same section as D, enlarged to show some detail of cell size and arrangement. F–H. Growing point of poisoned embryo. Note the broadening of leaf primordia and of growing point. Compare with B and C. I–J. Enlarged detail of tips of poisoned seedlings, showing enlarged cells and hypertrophied primordia. K–L. Cross sections of poisoned seedlings showing enlarged cells and irregular outlines of hypertrophied tissues. M. Growing point and greatly hypertrophied leaf primordia of poisoned seedling. N–O. Multinucleate cells from hypertrophied leaf primordia. The nuclei vary from very small ones to "giant nuclei." The former contain less than the normal chromosome complement ($n = 10$). The giant nuclei are polyploid. A. $\times 10$; B–D, F–H, K, $\times 70$; E, I, J, L, M, $\times 135$; N, O, $\times 300$.

cells, tissues and organs in proportion to the severity of the gross external symptoms. The first indications of histological abnormality in the plumule were found to occur in the older leaf primordia, in the form of isolated areas of enlarged cells (Fig. 2, I). At this stage the apical meristem and the younger leaf primordia are normal as to cell size and organization. As a rule, hypertrophy begins near the end of a primordium and progresses downward, the effect becoming evident in surface cells rather than in the cells of the interior of an organ (Fig. 2, J). Hypertrophic activity eventually spreads to the youngest leaf primordia and, finally, to the apical meristem. The formation of new cells and new leaf primordia ceases, the existing cells continuing their excessive, irregular enlargement. As the result of this activity, elongation of the axis and organs ceases, but these structures become greatly thickened. The outlines of the axis and leaf primordia exhibit irregular lobes and crenations (Fig. 2, F, I–M) suggestive of wound callus or certain types of gall. This resemblance is superficial, for the former types of wound hypertrophy are characterized by hyperplasia, whereas the hypertrophy of corn seedlings presents the more uncommon condition of hypertrophy without cell multiplication. The absence of hyperplasia is true only in the sense that cell multiplication does not accompany nuclear division.

The cells of the hypertrophied tissues of corn seedlings were found to be multinucleate. The number of nuclei in a cell varies from one to more than ten. Where many nuclei are present in a cell, the number is difficult to determine with certainty because the nuclei are clustered. In general, the larger cells contain more nuclei than the smaller cells. The nuclei of hypertrophied tissues, and in fact within a multinucleate cell, vary greatly in size and chromatin content. The diameters of small and large nuclei may be in a ratio as large as 1:20. The large nuclei frequently exhibit lobes (Fig. 2, N, O). Late prophase figures (Fig. 1, D) show that the several nuclei in a cell undergo independent, though usually simultaneous, mitosis.

The "giant nuclei" are clearly polyploid. The late metaphase figure (Fig. 1, B) has at least 100 chromosomes, or perhaps fragments. The chromosome group in figure 1, C, contains well over 200 chromosomes. Anaphase figures show an irregular multipolar separation of chormosomes, resulting in the formation of some small nuclei containing a few chromosomes, and the reincorporation of most of the numerous chromosomes in one or more polyploid giant nuclei. Cell wall formation appears to be inhibited, although spindle fibers and fragmentary wall plates are discernible. The formation of aneuploid and polyploid nuclei, and of multinucleate cells, is clearly the result of abnormal and incomplete mitosis.

The mechanism of these abnormal mitoses resembles various features of

the numerous cases cited by Kostoff and Kendall[2] and by Politzer,[3] but unlike many known cases, the abnormality in poisoned corn seedlings is most active in the meristems and primordia. Varying degrees of hypertrophy are observable in poisoned seedlings and there is a possibility of recovery, or perhaps survival to maturity. If recovery, and the persistence of the induced polyploidy can be demonstrated by experiments now in progress, this method of inducing polyploidy may be well worth the attention of plant breeders.

SUMMARY

An anatomical study was made of the hypertrophy produced in seedlings of corn by treatment with ethyl mercury phosphate.

The leaf primordia become much thickened, and develop irregular crenations and lobes.

In the leaf primordia and apical meristem of the plumule, cell division is inhibited; the existing cells undergo very great enlargement.

Cells of the hypertrophied organs become multinucleate, containing nuclei that range in size from minute "micronuclei" to very large "giant nuclei." The latter are polyploid.

The multinucleate condition and the formation of micronuclei and giant nuclei are the result of abnormal, incomplete mitosis.

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[2] Kostoff, D., and J. Kendall. Studies on plant tumors and polyploidy produced by bacteria and other agents. Arch. Mikrobiol. 4: 487–508. 1933.

[3] Politzer, Georg. Pathologie der Mitose. 238 pp. Gebrüder Borntraeger, Berlin. 1934.

SCLEROTINIA ROT OF IRISH POTATOES

A. H. EDDINS

(Accepted for publication June 30, 1936)

INTRODUCTION

A sclerotium disease of Irish potatoes caused by *Sclerotinia sclerotiorum* (Lib.) Massee was reported first from Ireland by Pethybridge in 1910 (4, p. 241–256). Lachaine (3) reported that it was present in New Brunswick, Canada, in 1922, but his photographs show that the sclerotia resemble those of *S. minor* Jagger more closely than those of *S. sclerotiorum*. Lachaine (3) also recorded the occurrence of the disease in England, Scotland, and Australia, and Brien (2) found it in New Zealand. In the United States, the disease has been reported from New York and Florida (1), Washington,[1] and Montana (6). *Sclerotinia sclerotiorum* also attacks many other plants (2, 6) and is one of the most important fungi causing decay of vegetables in transit (5).

THE DISEASE

Aside from Pethybridge's report that the disease was important in Ireland (4), no reports indicate that sclerotinia rot has caused any particular loss of potatoes in any locality, except Hastings, Florida, where it did some damage in 1933 and was very prevalent in 1934. During that year it killed 15 to 70 per cent of the plants in several fields, totaling 120 acres, and caused a 25 per cent reduction in yield in the most severely affected fields. There was, however, very little loss in this area in 1935.

The hosts of *Sclerotinia sclerotiorum*, as listed by Young (6), include many wild and cultivated plants that are widely distributed throughout the United States. At Hastings, Florida, plants found attacked by the fungus in and near potato fields are as follows: cabbage, *Brassica oleraceae* var. *capitata* L.; tomato, *Lycopersicon lycopersicon* (L.) Karst.; ragweed, *Ambrosia elatior* L.; sow thistle, *Sonchus oleraceus* L.; calendula, *Calendula officinalis* L.; fireweed, *Erechtites hieracifolia* L. Raf.; and water cress, *Radicula obtusa* Nutt. Green. The last 3 plants have not been previously reported as hosts of the fungus.

The fungus attacks the tops of potato plants, but does not infect the tubers. The main stem may be attacked at any point, but it usually is invaded at the soil line. The first sign of the disease on the stem is the appearance of a water-soaked lesion, followed by the development of white mycelium of the fungus on the surface. The epidermis in the infected area dies and turns brown as the lesions become 2 to 6 inches long. All except the

[1] U. S. Dept. Agr. Bur. Plant Indus. Reptr. Sup. 86: 47. 1935. [Mimeographed].

lignified tissues of the invaded parts are destroyed, and the interior of the stem becomes packed with mycelium and black sclerotia. The plant wilts when the water-conducting tissues are destroyed and finally collapses and dies. Any of the branches and leaves of the plant may become infected and the white mycelium is evident on the parts affected. The sclerotia, which are formed on the surface of the leaves and branches and inside the main stem and large branches, are of irregular shape and are $\frac{1}{8}$ to 1 inch long. The symptoms of the disease are illustrated in figure 1.

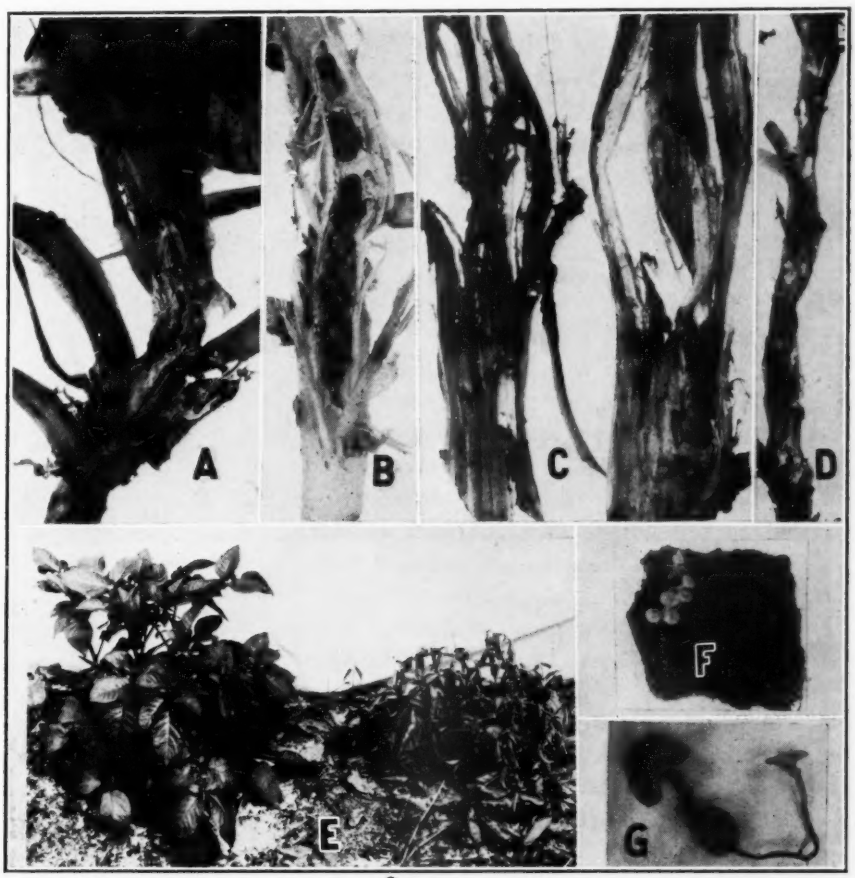

FIG. 1. Sclerotinia rot of potato. A. Lesion on lower part of stem. B. Stem spread open to show sclerotia. C. Shredded stem with parenchymatous tissue destroyed by the fungus. D. The fungus growing on surface of diseased branch. E. Healthy plant (left) and a plant wilted by sclerotinia rot of main stem. F. Six apothecia produced by one sclerotium. G. One sclerotium with two apothecia formed under field conditions.

Cool, rainy weather in March and April has been most favorable for the development of the disease in the Hastings, Fla., section. Such conditions prevailed in 1933 and 1934, when the disease was severe. In 1935, when the latter part of the growing season was hot and dry, it caused very little loss. Fogs and heavy dews also have been favorable. Furthermore, the greatest damage has occurred in potatoes on low ground that had remained wet for a prolonged period. The disease becomes most severe near the end of the growing period, when the dense foliage of the plants provides shade that favors growth of the fungus on the lower branches and leaves.

CAUSAL ORGANISM

Appearance of the Apothecia: In 1935, apothecia of *Sclerotinia sclerotiorum* were first observed in the field on February 8, 11 days prior to the appearance of the disease on the potato plants. The apothecia grew under the shade of the plants and in other locations in and between the rows. From 1 to 6 apothecia were seen arising from a single sclerotium (Fig. 1, F and G). The apothecial cups were well-expanded and measured from 1.5 to 10.0 mm. in diameter; the stipes varied from 2.5 to 24.0 mm. in length.

Infection: Infection is caused by ascospores, which germinate and invade potato stems and leaves with which they come in contact, and by mycelium, which comes in contact with healthy parts of the plant, as it grows on the soil and diseased potato stems and leaves. The disease is most severe on the parts of the plant nearest the ground, but some plants are affected only in their tops, indicating that infection in such cases was directly due to the ascospores, a fact proved by placing a potato plant, which had been grown in a pail, under a bell jar and by suspending apothecia, obtained from the field, inside the jar. The disease appeared first in the top of the plant nearest the apothecia that discharged the ascospores. The plant finally was killed and sclerotia were produced on the leaves and branches and inside the stem.

Cultural Characteristics and Pathogenicity of Sclerotinia sclerotiorum, S. minor, and S. intermedia.[2] When *S. sclerotiorum, S. intermedia* Ramsey and *S. minor* Jagger were grown in Petri plates on hard potato-dextrose agar, *S. sclerotiorum* formed mycelium more abundantly and produced larger, but fewer, sclerotia than either of the other species; *S. minor* produced the scantiest growth of mycelium and the smallest but most abundant sclerotia (Fig. 2).

Mycelial cultures of each of the 3 species, grown on potato-dextrose agar, were used to inoculate potato plants, kept in a moist chamber, by placing the mycelium of each species in contact with the nonwounded leaves and branches of each of 20 plants. *S. sclerotiorum* infected all of the 20 plants

[2] The culture of *Sclerotinia intermedia* was obtained from Dr. G. B. Ramsey, University of Chicago, Chicago, Ill., and *S. minor* from Dr. H. H. Whetzel, Cornell University, Ithaca, New York.

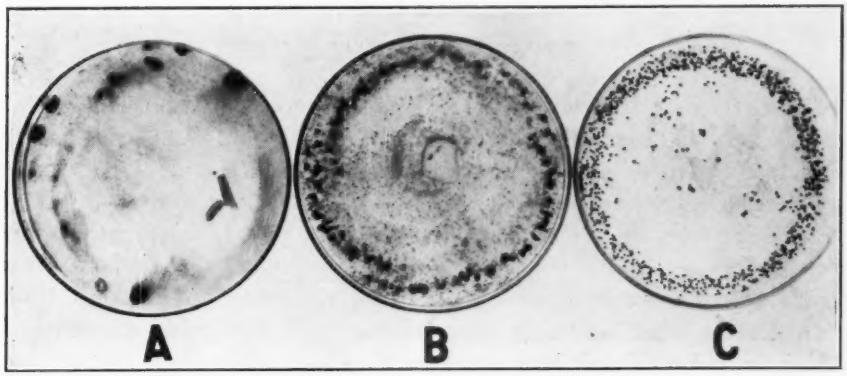

FIG. 2. Petri plate cultures of three species of Sclerotinia, grown for 30 days on potato-dextrose agar. A. *S. sclerotiorum.* B. *S. intermedia.* C. *S. minor.*

inoculated, and *S. minor* infected 11; symptoms of the diseases produced by both species could be distinguished only by comparing the size of the sclerotia of the 2 organisms from the diseased parts of the plants. *S. intermedia* did not infect the plants and formed no sclerotia.

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PHYTOPATHOLOGICAL NOTES

The Possibility of Ribes Infection by Aeciospores of Cronartium ribicola at Temperatures above 19° C.—The minimum, optimum, and maximum temperatures for the germination of aeciospores of *Cronartium ribicola* Fischer, in water, have been reported as 5°, 12°, and 19° C., respectively.[1] While conducting classes in culture methods during the past few years, the writer noted that fresh aeciospores of this fungus germinated readily in water at room temperature (approximately 21° C.). The writer also obtained infection of *Ribes rotundifolium* by inoculating a potted bush with aeciospores of *C. ribicola* and placing it in a damp chamber, where the temperature never dropped below 21° C. Since the blister rust fungus is gradually advancing into regions where its ability to cause infection of Ribes species at temperatures above 19° C. may prove to be of importance in blister rust control, it was deemed worthwhile to determine the highest temperatures permitting aeciospore germination.

On May 9, 1936, living bark with mature aecia of *Cronartium ribicola* was removed from each of 5 infected trees of *Pinus strobus*, growing in the Pack Demonstration Forest at Warrensburg, New York. The pieces of bark were placed in separate containers and, the following day, were brought to Syracuse, New York, where they were left, uncovered, in a laboratory for 48 hours. Examination of the spores at the end of that period disclosed no pregermination. On May 12, suspensions of aeciospores in distilled water were made in separate culture tubes from each of the 5 cankers. By means of a wire loop, drops of each spore suspension were drawn across the surface of solid water agar contained in Petri dishes. The free water was absorbed by the agar, and thus the aeciospores were brought into direct contact with the surface of the agar medium, which supplied sufficient moisture to permit spore germination. The Petri dishes were then covered and separated into 10 groups of 5 cultures each, such that each group contained 1 culture of spores originating from each of the 5 cankers. The cultures were placed in thermostatically controlled incubators with the exception of 1 set of cultures, which was placed in a container out-of-doors. The temperatures at which the cultures were incubated included those temperatures determined by Doran to be the optimum and maximum for aeciospore germination. The incubation temperatures were recorded on thermometers, except in the case of the out-door cultures, for which a standard thermograph was employed.

The cultures were examined at the end of 12 and 18 hours. The percentage of germination was approximately the same for the spores from each of the 5 cankers; hence the results were not recorded separately for the

[1] Doran, W. L. The minimum, optimum, and maximum temperatures of spore germination in some Uredinales. Phytopath. **9**: 391–402. 1919.

TABLE 1.—*The germination of aeciospores of Cronartium ribicola at different temperatures*

Temperature °C.	At the end of 12 hours			At the end of 18 hours				
	Spores counted	Germination	Maximum length of germ tube	Spores counted	Germination	Maximum length of germ tube	Average length of germ tube	Infection index[c]
	No.	*Per cent*	μ	*No.*	*Per cent*	μ	μ	
Out-door temperature[a]	500	83	732	500	83	1098	915	8
12[b]	500	79	475	500	83	732	658	5
18–19	500	71	732	500	73	732	549	4
20–21	1000	72	534	1000	74	732	549	4
22–23	500	70	366	500	74	732	549	4
24–25	500	75	732	500	78	732	549	4
26–27	500	40	732	500	66	732	439	3
28	500	28	534	500	31	732	439	1
29	500	0	.....	500	0	.....	.....	.....
30	500	0	.....	500	0	.....	.....	.....

[a] During the first 12 hours the temperature dropped steadily from 21° C. to 12° C. During the remaining 8 hours it ranged between 11° C. and 12° C.

[b] Optimum temperature as recorded by Doran.

[c] Per cent germination × average length of germ tubes. The results are divided by 10,000 and given in round numbers.

individual cankers. The resulting data are shown in table 1. Of particular significance are the facts that the percentage of germination decreased less rapidly in relation to increased temperatures than was found to be the case by Doran; and the maximum temperature for the germination of aeciospores was 28° C. instead of 19° C. Although aeciospores germinated at 28° C., the majority of the young germ tubes soon became vacuolate and at the end of 18 hours most of them appeared empty and apparently dead. The length of the germ tubes was measured but, because they were somewhat curved and twisted, it was impossible to obtain their exact length. However, the lengths recorded are approximately correct. After 18 hours, the aeciospores incubated at out-of-door temperatures averaged somewhat longer germ tubes than those spores incubated within a narrow temperature range. No explanation is advanced for this phenomenon. In nature, however, spores are normally subjected to fluctuating temperatures during germination.

The results obtained are significant in determining the relative possibility of Ribes infection from aeciospores of *Cronartium ribicola* at various tem-

peratures. When considering the possibility of infection, the length of the germ tubes at the end of a definite period is as important as the ability of the spores to germinate. If the germ tubes are not sufficiently long to extend from the spores into the mesophyll tissue, infection will not occur. Measurements taken on leaves and sections of leaves from 4 species of Ribes showed that half the average distance between the stomata plus the distance from the stomata to the mesophyll tissue was less than 400 μ. Thus a relative index to the possibility of infection at different temperatures at the end of 18 hours was obtained by multiplying the percentage of germination by the average length of the germ tubes. The infection index (Table 1), calculated from the data obtained in this study, indicates that infection may occur at temperatures as high as 28° C. within 18 hours, but that it is more apt to occur at the lowest temperatures used in this experiment.—RAY R. HIRT, Department of Forest Botany and Pathology, New York State College of Forestry, Syracuse, N. Y.

A Bacterial Wilt and Soft Rot of the Potato in Maine.—Within recent years the writer's attention has been called to a soft rot and wilt of potatoes in Aroostook County, Maine. The disease was first noted by the writer in 1932 in a field where 20 per cent of the tubers were decayed by a soft rot. A former seed-potato inspector, however, has stated that it was present in some fields of Maine several years prior to 1932. Since 1932 it has appeared annually in certain localities in Aroostook County.

Up to the present the disease has been most commonly observed in the vicinity of Fort Fairfield. It also has been found farther north toward New Sweden, and specimens have been received from Presque Isle. B. Baribeau has reported a very similar disease from the Province of Quebec, Canada.[1]

This soft rot of the potato apparently is not yet generally distributed in Aroostook County. The losses in a few individual fields, however, have been large. One small field in 1934 showed 30 per cent of the plants affected and fully one-third of the crop decayed, either in the field or in the bin. In Maine it generally is noted in late summer, when the tubers are well formed. The disease was present to a less extent on several other farms in the same vicinity. In 1935 it was again present on one of these farms, causing a 20 per cent loss in portions of a field and also some damage in other fields in the same vicinity. The writer has observed it mostly on more or less poorly drained soil. Some growers, however, maintain that it frequently occurs on the higher and well-drained soils.

It has been observed most commonly on the Green Mountain variety, but was found also on the Irish Cobbler and Katahdin varieties.

[1] Baribeau, B. Geographical distribution of bacterial blight of potatoes in Quebec. Ann. Rep. Quebec Soc. Prot. Plants 27 (1934/35): 80–83. Illus. (map). 1935.

The first aboveground evidence of the disease is a wilting of leaves and individual stalks (Fig. 1). The leaves on the wilted stems become chlorotic

FIG. 1. Green Mountain potato plant showing typical wilt symptoms in the field. Note healthy stalk on the right.

and gradually die. They sometimes show marginal necrosis, and the vascular bundles of the stems appear more or less discolored. On digging, it is noted that the tubers originating from the wilted stems are decayed. The decay appears to begin in the region of the vascular system and thence extends into the pith, causing a white or cream-color rot (Fig. 2). Often the entire center of the tuber disintegrates, leaving the mere shell. These hollow tubers frequently are found in a bin of potatoes from an affected field.

The tubers from affected plants, if harvested before the rot sets in, often show a characteristic cracking (Fig. 2).

The disease is perpetuated through the seed tubers. In 1935 apparently healthy tubers were harvested from diseased plants. Most of these tubers, although apparently disease-free, decayed in storage. Thirty-six plants, however, were raised from the seed stock that survived, 5 of which showed typical wilt symptoms. Most of the 36 plants developed tubers with characteristic cracking when harvested in the fall. Some growers insist that

FIG. 2. A. Two tubers showing typical cracking. Healthy tuber on the left. B. Halved tubers showing different stages of vascular discoloration and decay.

their potatoes acquired the disease through seed stock introduced from other farms.

The writer has attempted to identify the organism responsible for this disease. A bacterium having the salient physiological and morphological characteristics of *Bacillus carotovorous* L. R. Jones has been most commonly isolated. This organism, isolated from wilt-diseased plants, is capable of causing a white soft rot of potato tubers, but in no case has it produced the symptoms of the disease in question, on inoculation, nor has it been found capable of causing blackleg when introduced into potato stems. Another organism very similar to the soft rot has been isolated that is characterized as a rapid gas former on the various sugar media. Several other bacteria (not related to the soft-rot bacteria) have been secured from the tissue of diseased potatoes, but in no case have inoculations with these organisms produced the symptoms of this disease.

Growers are of the opinion that use of new seed stocks has eliminated the disease from their respective farms.—REINER BONDE, Maine Agricultural Experiment Station, Orono, Maine.

Comparative Studies on Cultures of **Phytomonas lactucae-scariolae,** ***n. sp. and Phytomonas pruni.***—A leaf-spot disease of wild lettuce, *Lactuca scariola,* observed by the writers on June 25, 1929, at Urbana, Illinois, appeared to be of bacterial origin from miscroscopic examinations. Sectioned and stained material demonstrated the location, as well as the presence, of bacteria in the infected tissue. Since no reference to this disease was found in the literature, and, since the organism in culture appeared similar to *Phytomonas pruni* (E. F. Smith) Bergey *et al.,* 1930, a comparative study of morphological and biochemical properties of the two organisms was carried out under identical cultural conditions following the Manual of Methods for Pure Culture Study of Bacteria prepared by the Society of American Bacteriologists. These results and a description of the disease on wild lettuce are here reported.

The lesions on wild lettuce, on first appearance, are circular and water-soaked, but they develop rapidly into decidedly angular spots. The mature lesions are from 2 to 4 mm. in diameter and the tissues are shrunken and light brown. A distinguishing character of the bacterial spots from other lesions is the glistening oily appearance given the under, and sometimes upper, surface by the exudate produced from bacterial growth in the tissue. Free-hand sections of the spots showed bacteria oozing from the margin of diseased and healthy tissues, when examined miscroscopically in water mounts.

The organism on wild lettuce was repeatedly isolated from lesions by the dilution-plate method, and produced typical symptoms 5 days after atomizing 6-day-old cultures to healthy wild-lettuce plants kept in moist chambers 36 hours prior to and 24 hours after inoculation. Reisolations yielded typical colonies of the pathogen. Pathogenicity of *Phytomonas pruni* has been determined previously from pure cultures isolated from diseased tissue. Inoculations with cultures of *P. lactucae-scariolae* to peach foliage and green shoots and with culture of *P. pruni* to wild lettuce were negative in all cases, showing different pathogenicity for each organism. The morphological and biochemical properties and cultural features of the two organisms were indistinguishable in all measurements. Although the bacteria differed only in their pathogenicity the evidence seems inadequate to consider these as related strains of bacteria. For this reason, for the custom of naming bacteria according to the host infected, and to avoid confusion with *Bacillus lactucae* Voglino[1] the name **Phytomonas lactucae-scariolae,** n. sp. is proposed for the organism pathogenic to wild lettuce. The results are given in the technical descriptions.

Phytomonas lactucae-scariolae, n. sp. is a short rod, 1.0 to 1.5 μ long

[1] Elliott, Charlotte. Manual of bacterial plant pathogens, 1930, Baltimore, The Williams Wilkins Company.

and 0.5 to 1.0 μ wide; motile by 1 and 2 polar flagella; occurs singly, in pairs, and in short chains, but mostly in pairs; is capsulate, but forms no endospores or granules; is Gram-negative and not acid-fast; forms round, entire, finely granular, amber-yellow colonies on dextrose agar; clouds bouillon, and forms a yellowish rim, but no pellicle; has slight diastatic activity; liquefies gelatin slowly after 10 days; reduces nitrates, but does not produce hydrogen sulphide; produces very slight acid reaction and causes peptonization in litmus milk, with reduction of litmus and slight coagulation; does not produce gas from xylose, rhamnose, glucose, mannose, galactose, fructose, maltose, sucrose, rhaminose, raffinose, dextrin, inulin, glycerol, mannitol, sorbitol, ducitol, or salicin; is aerobic; optimum, minimum, and maximum temperatures and H-ion concentrations for growth are 25, 7, and 35° C. and pH 7.5, pH 4.8, and pH 11.0, respectively; causes dark irregular spots on leaves of wild lettuce, *Lactuca scariola*, but is non-pathogenic to cultivated lettuce, *Lactuca sativa*.

Phytomonas pruni (E. F. Smith) Bergey *et al.*, 1930, having the same morphological and biochemical characteristics as *P. lactucae-scariolae*, causes irregular lesions on leaves, green shoots, and fruits of peach, *Amygdalus persica*, but is non-pathogenic to wild lettuce. These properties are similar to those listed by Elliott (2) for the organism.—H. H. THORNBERRY[2] AND H. W. ANDERSON, Department of Horticulture, University of Illinois, Urbana, Illinois.

Control of Peach Leaf Curl by Autumn Applications of Various Fungicides.—While making tests of various fungicides for the control of peach blight (*Coryneum beijerinckii* Oud.) it became necessary to determine whether the spray applied for this disease (an application after the leaves are off, usually between November 15 and December 15), also would prevent leaf curl [*Taphrina deformans* (Fckl.) Tul]. Although the time most frequently set for applying the leaf curl spray is the early spring before the buds begin to open, there is evidence[1,2,3] that both Bordeaux mixture and lime-sulphur applied in the autumn adequately controls the disease. Recently, however, Fitzpatrick,[4] working in Ontario, tested the efficiency of lime-sulphur applied at different times in autumn and spring. He found that autumn sprays gave poorer control of leaf curl than those applied in early spring. If the autumn spray should fail to prevent leaf curl in California,

[2] Department of Agronomy, University of Kentucky, Lexington, Ky.

[1] Smith, R. E. California peach blight. California Agr. Expt. Sta. Bull. 191. 1907.

[2] Reddick, D. and L. A. Toan. Fall spraying for peach leaf curl. New York (Cornell) Agr. Expt. Sta. Circ. 31. 1915.

[3] Berkeley, G. H. Fall spraying for peach leaf curl. Canad. Hort. 47: 245. 1924.

[4] Fitzpatrick, R. E. The life history and parasitism of *Taphrina deformans*. Sci. Agr. 14: 305–326. 1934.

a second application in early spring would be necessary, since fungicides applied much later than December 15 fail, in many years, to control peach blight.

With these points in mind, plots of from 16 to 20 trees were sprayed at different times and with different materials during the autumn and spring of 1935–36. Results taken on 2 different dates in the spring appear in table 1.

TABLE 1.—*Results of spraying for the control of leaf curl on Elberta peach trees, 1935–36*

Treatment[a]	Leaves infected on March 13	Leaves infected on April 7
	per cent	*per cent*
Nonsprayed, Plot 1	42	46
" Plot 2	33	43
" Plot 3	29	36
" Plot 4	30	45
Bordeaux mixture, 2–5–50, Oct. 24	0.7	0.6
" " 5–5–50, Oct. 24	0.2	0.2
" " 5–5–50, Nov. 29	0.1	0.1
" " 5–5–50, Jan. 29	0.4	0.1
" " 5–5–50 + oil, Nov. 29	0.2	0.3
Lime-sulphur, 4–50, Nov. 29	0.5	1.0
Basic copper sulphate, 3–50, Nov. 29	0.1	0.3
Copper ammonium silicate, 3–50, Nov. 29	2.2	13.0

[a] All Bordeaux mixture was made with unslaked lime.

Bordeaux mixture + oil = a dormant oil emulsion added to Bordeaux mixture at the rate of 4 gallons per 100 gallons of spray.

Lime-sulphur = a standard brand of the liquid material.

Basic copper sulphate = Basicop, a material containing 52 per cent metallic copper, with a spreader, recommended by the manufacturer, added at the rate of ½ pint per 100 gallons of spray.

Copper ammonium silicate = Coposil, a material containing 22 per cent metallic copper, with one gallon of a dormant oil emulsion added to each 100 gallons of spray.

The first spring infection undoubtedly was initiated during a prolonged rain, which lasted until the tips of the first two or three leaves were exposed. A second wave of infection, appearing between March 13 and April 7, probably was initiated during a series of rains between March 26 and April 4.

Bordeaux mixture, 5–5–50, gave excellent control, whether applied on October 18, November 29, or January 26 (about three weeks before buds began to swell). Bordeaux, 2–5–50, Bordeaux-oil, and Basicop applied in autumn also gave good control. Coposil, on the other hand, did not prevent the disease so efficiently as did the other materials. Although no spring application of lime-sulphur was made, control could not have been significantly better than that afforded by the autumn application.

Fitzpatrick's studies of the fungus afford an explanation of the difference between the results of his tests and those of the present tests. In the first place, Fitzpatrick sprayed single branches, while in the present test the entire tree was sprayed. Fitzpatrick maintained that sprout conidia, produced by budding of the ascospores, are spread throughout the tree by rain. If, therefore, all of the sprout conidia in the tree are not killed by the fungicide, they are likely to be carried to sprayed branches after the spray is washed away by rain. The reason his autumn application failed to control the disease was because the conidia from nonsprayed branches spread to the sprayed ones after the spray coating weathered away. This spring application gave good control because the spray coating, retaining its effectiveness until the leaves appeared, prevented the conidia from reaching and infecting the leaves. In the present tests, on the other hand, the entire tree having been sprayed, there remained in the tree no large amount of viable inoculum to spread to the leaves when they appeared. There was, of course, the possibility of lateral drifts of conida from the other trees, but no evidence of this was seen in autumn-sprayed trees adjacent to nonsprayed plots.—E. E. WILSON, Division of Plant Pathology, Branch of the College of Agriculture, Davis, California.

Blooming of Potatoes as Influenced by Pyrethrum Dust.—In connection with studies on the stimulation of potato plants by Bordeaux mixture it was noted in 1935 that pyrethrum dust (a mixture of 25 pounds of pyrethrum[1] and 75 of Celite[2]) used as an insecticide had a decided effect on the foliage development of the plants. Further tests were conducted in 1936 to determine the merits of this material to induce increased foliage weight, as well as to continue the study of the stimulating effect of Bordeaux mixture on plants, where the insects were largely controlled by pyrethrum. The experiments have not yet been completed (September 1, 1936), but a striking phenomenon, that of increased blooming, has occurred in the pyrethrum-treated plants. Under the rather poor growing conditions in the 1935 field, no blossoming occurred on any of the plots.

The total number of blossom clusters, blooming and not blooming, was recorded in 1936 on a 100-plant basis (Table 1). Two noteworthy tendencies may be noted. First, pyrethrum applied in addition to Bordeaux mixture practically doubled the number of blossom clusters, and an even greater increase took place on the plants that were not sprayed with Bordeaux mixture, that is, if pyrethrum alone was used. Secondly, more than half the total blossom clusters opened their flowers when pyrethrum dust

[1] A pyrethrum dust, "Powco A," procured from John Powell and Co., Inc.

[2] A relatively inert powder consisting chiefly of silica and other inert ingredients procured from the Johns Manville Co.

TABLE 1.—*Number of blossom clusters on 100 plants under different dust and spray treatments*

	Bordeaux mix. alone			Unsprayed	Bordeaux mix. + pyrethrum dust			Pyrethrum dust alone
	1[a]	2[a]	3[a]		1[a]	2[a]	3[a]	
No. of blossom clusters not blooming	143	162	108	95	116	121	114	137
No. of blossom clusters blooming	16	20	26	28	141	156	141	162
Total No. of blossom clusters	159	182	134	123	257	277	255	299

[a] Different spray schedules.

was applied. When Bordeaux mixture alone was used only a small proportion of the flowers opened. A slightly higher number of flowers blooming was found in the unsprayed than in the Bordeaux sprayed plants. This has been commonly noted during a number of years in these experiments.

The nature of these phenomena is not yet understood. It is believed that something other than insect control is involved. Whether pyrethrum dust acts as a stimulant to the growth of the plant, remains to be proved. Experimental evidence concerning foliage (and tuber) weights of plants treated with pyrethrum in addition to copper suggests this latter possibility (unpublished data).—E. O. MADER ANND E. C. UDEY, Department of Plant Pathology, Cornell University.

Physiologic Races of Snapdragon Rust.[1]—The heavy natural rust infection occurring on rust-resistant snapdragons[2,3,4] in certain coastal regions of California in 1936 indicated that a hitherto-nonrecorded race of the rust (*Puccinia antirrhini* Diet. and Holw.) may have appeared. To test this possibility rust-susceptible and rust-resistant plants were inoculated with rust from rust-resistant plants grown in several places in California, and with rust from rust-susceptible plants grown in Berkeley, where rust-resistant plants remained rust-free during the 1936 season.

Because of the unavailability of rust-free, rust-resistant, and rust-susceptible plants, and because of the convenience and reliability of the dish-culture method,[5] excised leaves were used for making the tests. Two rust-

[1] The writer wishes to acknowledge the cooperation of Dr. D. R. Porter and Mr. C. O. Blodgett in securing some of the cultures of rust and snapdragons used in this study.

[2] Emsweller, S. L. and Jones, H. A. The inheritance of resistance to rust in the snapdragon. Hilgardia (California Agr. Expt. Sta.) 8: 197–211. 1934.

[3] Mains, E. B. Studies in rust resistance. Jour. Heredity 17: 313–325. 1926.

[4] Mains, E. B. Rust resistance in Antirrhinum. Phytopath. 25: 977–991. 1935.

[5] Yarwood, C. E. The comparative behavior of four clover-leaf parasites on excised leaves. Phytopath. 24: 797–806. 1934.

TABLE 1.—*Differentiation of physiologic forms of snapdragon rust on excised leaves on 5 per cent sucrose solution in Petri dishes*

Host plant number	Previous history of plants in regard to rust resistance	Inoculated with physiologic race 1 of snapdragon rust		Inoculated with physiologic race 2 of snapdragon rust	
		Number of leaves inoculated	Total number of rust pustules	Number of leaves inoculated	Total number of rust pustules
17	rust-susceptible	11	209	11	70
18	rust-susceptible	10	198	11	96
1	rust-resistant prior to 1936	18	0	18	389
2	rust-resistant prior to 1936	13	0	12	307

susceptible but rust-free greenhouse plants and several rust-free and rust-resistant plants growing beside heavily infected rust-susceptible plants, outdoors at Berkeley, were used as host material. Vigorous leaves were distributed in Petri dishes with their dorsal surfaces upward, 2 Petri dishes of leaves from each test plant. One Petri dish of leaves from each plant was dusted with urediospores from rust-susceptible plants grown in Berkeley, and the other with urediospores from rust-resistant plants obtained in another locality. The dusted leaves were atomized with water. Five per cent sucrose solution was placed in each dish, and the inoculated leaves floating on the sugar solution were incubated in diffuse light at room tem-

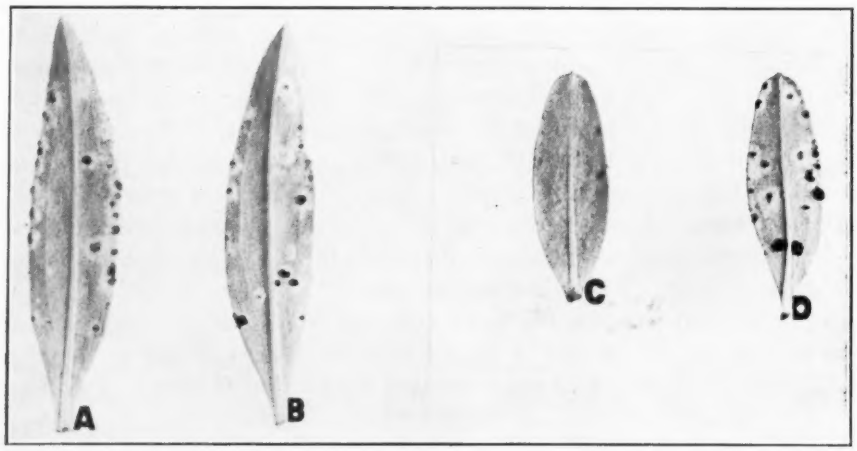

FIG. 1. Differentiation of physiologic races of snapdragon rust on excised leaves on 5 per cent sucrose solution in Petri dishes. Photographed 13 days after inoculation. A. Rust-susceptible plant inoculated with race 1. B. Rust-susceptible plant inoculated with race 2. C. Rust-resistant plant inoculated with race 1. D. Rust-resistant plant inoculated with race 2.

perature (19–23° C.). Rust pustules were evident in about 7 days; many had broken through the epidermis in 10 days. Representative results in the first test, 13 days after inoculation, are represented in table 1 and figure 1.

The leaves of rust-susceptible plants became heavily infected with all rust collections, but the leaves of rust-resistant plants became infected only with the rust from rust-resistant plants. Rust-resistant snapdragons were immune from rust collected at Berkeley in 5 tests, and to rust occurring at Davis and Sacramento in 1 test. The susceptibility of rust-resistant snapdragons to rust occurring 5 other localities in coastal California has been demonstrated in 5 tests. These results clearly indicate that there are at least 2 races of *Puccinia antirrhini*. The rust to which the resistant plants are resistant will be termed physiologic race 1, and that infecting the rust-resistant selections will be termed physiologic race 2. Races 1 and 2 may each comprise several races of rust, as the writer did not work with pure cultures, but no evidence of further races was observed. Of 11 tested plants of different genetic lines of rust-resistant snapdragons, none have proved to be resistant to race 2.—CECIL E. YARWOOD, Division of Plant Pathology, University of California, Berkeley, California.

A Method of Inoculating Seed Barley with Black Loose Smut for Use in Studies on Physiologic Races.—In previously reported studies[1] on the black loose smut of barley (*Ustilago nigra* Tapke), the writer obtained high percentages of smutted heads as a result of blackening the seed with dry smut spores. Although effective and easy to apply, this method presents an inherent difficulty when used in physiologic race studies involving many different smut collections that must be prevented from mixing. The dry spores are so volitant that it is difficult or impossible to keep them confined, even by the most careful handling, in the preparation of inoculum by removing the spores from the smutted heads and in applying the smut dust to the seed. Furthermore, if the dusted seed is placed in envelopes and the latter are squeezed in handling, puffs of smut emerge from the corner vents of the envelopes and a further spread of the smut occurs.

To avoid this difficulty the writer has devised a wet method of preparing the inoculum. The latter is then applied to the seed by the spore-suspension method,[2] recently described for inoculating seed barley with covered smut. The method as used in recent studies of physiologic races of *Ustilago nigra* is as follows: Four loose smut heads are immersed in 750 cc. of

[1] Tapke, V. F. A study of the cause of variability in response of barley loose smut to control through seed treatment with surface disinfectants. Jour. Agr. Res. [U. S.] 51: 491–508. 1935.

[2] Tapke, V. F. An effective and easily applied method of inoculating seed barley with covered smut. Phytopath. 25: 1038–1039. 1935.

water in a 1-liter Erlenmeyer flask. By vigorously shaking the flask the spores are loosened from the heads and suspended in the water. The suspension is then poured into another vessel through a fine screen to remove the remnants of the smutted heads and any other extraneous matter. This spore suspension is then used to inoculate thoroughly dry seed, previously treated by the modified hot-water method for the prevention of loose or covered smuts. The fluid is poured over small lots of seed in shell vials until it rises about $\frac{3}{4}$ inch above the seed. The seed is vigorously shaken in this suspension for $\frac{1}{2}$ minute, then allowed to soak 15 minutes. The suspension then is decanted and the vials are inverted on clean pieces of blotting paper to absorb all free water. Next, the vials of moistened inoculated seed are packed, uncorked, in a tightly covered tin box floored with a wet blotter to maintain high humidity, and then incubated for 24 hours at 18–20° C. Lastly, the seed is transferred to small envelopes, crimped to remain wide open. where it is left 2 or 3 days, or until thoroughly dry. It is then ready to sow. Through the cooperation of the North Carolina Agricultural Experiment Station and the Division of Forage Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, a field planting of 25 varieties of winter barley, each inoculated by this method with 10 different collections of *U. nigra,* was made at Statesville, N. C., in the fall of 1935. Despite the severe winter of 1935–36, high percentages of smutted heads, reaching a maximum of 83 per cent were obtained in the spring of 1936. It is, therefore, evident that the method is highly effective. Moreover, it has the desirable feature of practically eliminating the hazard of mixing the smut collections, so troublesome in the dust method.—V. F. TAPKE, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

BOOK REVIEW

Boysen, Jensen P. *Growth Hormones in Plants.* Authorized English translation of Die Wuchsstofftheorie . . . Translated and revised by George S. Avery, Jr., and Paul R. Burkholder, with the collaboration of Harriet B. Creighton and Beatrice A. Scheer. 1st Ed. 268 pp. McGraw-Hill Book Company, New York and London. 1936. $3.50.

Bibliographies: pp. 221–253.

While this book is of primary importance to those interested in plant physiology, phytopathologists will find it interesting despite the fact that bacteria and fungi have not been included in the scope of the work. However, there is a fairly comprehensive list of references dealing with bios, folliculin, and other sex hormones, and those affecting the growth of fungi. The historical review is cleverly treated by means of sketches and explanatory notes. Throughout the book the distribution, detection, and quantitative determination of growth substances are demonstrated by the curvature responses of the coleoptile of Avena. These substances have been detected and in some cases have been isolated in chemically pure form from many sources, such as human urine, corn oil, malt, yeast, Aspergillus, Rhizopus, etc. In addition, numerous synthetic compounds have been shown to possess similar growth-inducing properties in various degrees of intensity. "Hormones" have been demonstrated in many green plants belonging to widely separated taxonomic groups, ranging from Avena to Sequoia, and from Lupinus to orchids. Numerous fungi, bacteria, and at least one alga, Valonia, have been shown to possess growth-inducing properties. The formation of such substances in fungi seems to be influenced by glucose peptone, glucose-ammonium tartrate, tryptophane, tyrosin, etc. While it is comparatively simple to demonstrate the presence of growth substances in plants, their transportation in the tissues presents certain difficulties. The translocation seems polar, but is abolished by anaesthesia. It is shown that no growth of shoots of higher plants can take place without the "hormones," yet the elongation of the roots is inhibited by these substances; this suggests a selective toxicity. It is possible that these growth substances are not used as "building stones" but as activators.

It seems that this growth substance is formed in expanding buds and leaves, whence it moves into other parts of the plant, exercising some control over growing parts, and under some conditions promoting callus tissues, tumors, and roots. Nastic responses, prolongation of growth period and prevention of petiole abscissions also have been shown to be influenced by auxins. Phototropic curvatures are influenced by the unequal displacement of growth substances, whereby the illuminated sides of the plant receive less

auxins than the shaded sides. Similarly, geotropic, traumatic, and thigmatic curvatures have been shown to be influenced by auxins.

The title of the book is perhaps a bit unfortunate, as the term hormone belongs to animal physiology and constitutes the product of specialized glands. Furthermore, at least some of the substances, treated by the author as hormones, may not be true auxins but toxic substances that irritate the plant into an excessive production of auxins. Looking upon the phenomenon of excessive callus, root, and tumor formation under the action of these alleged hormones, the plant pathologist can see a pathological condition induced by the presence of substances inimical to the plant. Witches'-broom, hairy root, tumors, galls, etc., are all too familiar examples of the action of bacteria and fungi upon their host plants. It is more logical to assume that such abnormal growths are the result of excessive production and concentration of auxins in an attempt to overcome the invader than to presume that the pathogen furnishes growth substances to the host plant. If such be the case, then we still have to isolate in chemically pure form the true growth substances found in green plants.—LEON H. LEONIAN, West Virginia University, Morgantown, West Virginia.